The Journal of Allergy

VOL. 28 MARCH, 1957 NO. 2

Original Articles

PRELIMINARY EVALUATION OF A NEW CORTICOSTEROID* IN ALLERGIC DISEASES

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THE use of the various corticosteroids in the treatment of allergic diseases during the past few years has provided a valuable addition to the armamentarium of the physician. Since the introduction of cortisone, subsequent analogues, such as hydrocortisone, prednisone, and prednisolone, have offered certain advantages. For instance, the use of prednisone and prednisolone has demonstrated that less sodium retention will occur with these compounds than with hydrocortisone or cortisone. The aim in the further improvement of corticosteroids is to find a compound which will produce the therapeutic effect desired without the undesirable side effects. Whether or not this will be possible remains to be proved.

This report deals with the use of a new synthetically prepared corticosteroid compound in the treatment of allergic diseases. Its chemical formula is 9 alpha-fluoro-16 alpha-hydroxyl-delta-l-hydrocortisone diacetate, and it has been given the generic name of triamcinolone.

The significant feature of this compound is the hydroxyl group at the 16 position. Animal assays[1] indicate that this compound is eighteen to thirty-six times more potent than hydrocortisone by the rat liver glycogen assay. In the adrenalectomized rat, this compound produced no sodium retention.

Hellman and associates[2] have reported on this compound and presented the results of metabolic studies and its use in rheumatoid arthritis. Their work indicates that triamcinolone possesses excellent antirheumatic properties and, at the usual therapeutic dose, produced little or no change in nitrogen, calcium, potassium, or sodium balance.

From the Institute of Allergy, Roosevelt Hospital, New York, N. Y.
Received for publication Jan. 17, 1957.
*Supplied by the Research Division of American Cyanamid Company, Lederle Laboratories, Pearl River, N. Y.

TABLE I. COMPARISON OF PREDNISONE AND PREDNISOLONE WITH TRIAMCINOLONE

DIAGNOSIS	NO. OF WEEKS TREATED		DOSAGE (MG. PER 24 HOURS)		BLOOD PRESSURE RESPONSE	WEIGHT CHANGE (POUNDS)		CLINICAL RESPONSE	SIDE EFFECTS
	P. & P.*	T.†	P. & P.	T.	T.	T.		T.	T.
1. Asthma	26	11	10	5	Same	Increased	3	Same	Increased appetite
2. Asthma	1	11	15	6	Same	Same		Same	0
3. Active TB Asthma	104	11	20	12	Decreased	Decreased	7½	Better	0
4. Asthma ASHD‡ (compensated) hypertension	104	11	5	3	Decreased	Same		Same	0
5. Asthma	26	11	10	6	Decreased	Increased	5	Same	Increased appetite
6. Asthma	26	11	10	6	Same	Decreased	1	Better	0
7. Asthma ASHD (compensated) hypertension	86	11	10	8	Same	Decreased		Same	0
8. Obesity Asthma	13	11	15	6	Decreased	Increased	2	Better	0
9. Asthma Anemia	35	11	10	4	Decreased	Increased	5	Same	0
10. Asthma	2	11	10	6	Decreased	Increased	4	Better	Increased appetite
11. Asthma Dermatitis	104	11	15	8	Decreased	Decreased	3½	Better	0
12. Asthma	8	6	10	6	Decreased	Decreased	4	Better	0
13. Asthma	8	11	10-15	8-9	Decreased	Decreased	10½	Better	0
14. Asthma Hypertension	104	8	15-20	12	Decreased	Decreased	9	Same	0
15. Perennial rhinitis	26	8	5	2	Same	Decreased	2	Same	0
16. Asthma	Corticotropin gel 18	4	40 units q 1-2 days	15	Decreased	Decreased	5½	Better	0
17. Asthma	6	5	10	3-4	Decreased	Decreased	3½	Same	0

*P. & P. = Prednisone and prednisolone.
†T. = Triamcinolone.
‡ASHD = Arteriosclerotic heart disease.

METHODS

Triamcinolone was administered in divided daily doses to twenty-six patients. This report will deal with seventeen of these patients, since the remaining nine patients have been maintained for less than four weeks. Of the seventeen patients, sixteen had bronchial asthma and one had perennial rhinitis. Twelve of these have been followed for eleven weeks and the other five for four to eight weeks. All patients except one were on prednisone or prednisolone for one week to 104 weeks, with doses varying from 5 to 10 mg. daily. One patient had been on corticotropin gel, 40 units daily or every other day for eighteen weeks. All patients had been receiving specific injection therapy for many years, as well as symptomatic drug therapy, without good control prior to the use of prednisone and prednisolone. Antibiotic therapy was given as necessary. All therapy was continued, except for prednisone and prednisolone or corticotropin gel, when the patients were transferred to triamcinolone. All the patients transferred to triamcinolone presented problem cases; although they were controlled on previous therapy, a few had side effects from steroid or corticotropin treatment. In evaluating our patients, we relied on changes in symptomatology, physical findings, blood pressure, weight, and vital capacity studies. Every patient was seen at least once a week.

RESULTS

Table I gives a résumé of the patients treated. Patients on prednisone and prednisolone had received treatment from one week to 104 weeks prior to triamcinolone. The dose of prednisone and prednisolone varied from 5 to 20 mg. per day, in divided doses. These doses were carefully arrived at by a study of each patient to achieve the best possible symptomatic control. The maintenance dose of triamcinolone used in bronchial asthma varied from 3 to 15 mg. per day, and was arrived at in the same manner.

On this maintenance dosage it was found that nine patients showed no improvement, while eight were symptomatically better. The patients classified as better were placed into this category because of objective and subjective signs of added benefit from the new steroid. The objective signs relied upon were fewer sibilant and sonorous râles and an increase in vital capacity. Subjective signs were less cough, wheezing, and dyspnea.

There was no increase in blood pressure in any patient. In twelve patients there was a reduction in blood pressure. Three of these had been hypertensive. Patient 4 was receiving hexamethonium and the blood pressure returned to levels that temporarily necessitated discontinuing the drug. In this patient the blood pressure fell from 206/100 to 136/79 during a nine-week period. Patient 16, who had received corticotropin gel, had a marked moon face and other signs of fluid retention which disappeared during treatment with the new steroid. This patient's blood pressure dropped from 205/105 to 154/86.

Eleven patients lost weight, although only one (No. 16) had had obvious edema. Patient 13 lost up to 10½ pounds over an eleven-week period,

but most of the weight loss was during the first seven to fourteen days of triamcinolone therapy. Our findings of loss of weight and disappearance of moon face are consistent with the findings of Hellman and associates.[2]

Of the five patients who gained weight, three had improved appetite and food intake, which probably accounted for the increase in weight as there was no evidence of salt or water retention. Patient 9 was markedly underweight and anemic prior to treatment and was placed on a high-calorie diet with vitamins and iron supplements. None of the patients developed signs of fluid retention as evidenced by moon face or ankle edema.

On a weight basis, triamcinolone was found to have approximately twice the clinical effectiveness of prednisone or prednisolone. There has been no evidence of gastrointestinal discomfort, hirsutism, facial roundness, acne, sleeplessness, or mental aberrations in any of the patients treated.

One patient with complicating dermatitis noted marked improvement in this condition. The patient with tuberculosis did not develop any flare-up of the tuberculous process as judged by clinical signs, symptoms, or x-ray findings. This was in conformity with results on previous steroid therapy. It should not be inferred that we routinely utilize steroid therapy in asthmatic patients with tuberculosis; however, in this case it was a necessary calculated risk.

The nine patients not included in the table have been followed for only a few weeks. None has shown an increase in weight or other signs of fluid retention or an increase in blood pressure. One patient had moon facies and a hypertension of 205/105 while on prednisolone, and in this patient the moon face disappeared and the blood pressure returned to normal.

COMMENTS

The results of this study show that triamcinolone is a potent antiallergic compound. Although the therapeutic doses administered in this series produced no significant untoward side effects, the capacity of triamcinolone to produce peptic ulcer, psychosis, and osteoporosis could not be properly evaluated in such short-term observations. As with any corticosteroid compound, the usual precautions should be adhered to, and the dose in each patient should be carefully titrated. It should be pointed out that it was not necessary to administer supplemental potassium. The sodium intake in patients during this study remained at the level previously maintained during prednisone and prednisolone administration. The use of any of the newer antiallergic steroids does not obviate the importance of sound allergic diagnosis and treatment.

SUMMARY

Sixteen patients with bronchial asthma and one with perennial rhinitis were treated for four to eleven weeks with triamcinolone. The compound was demonstrated to have a potent therapeutic effect about twice that of prednisone or prednisolone, on a weight basis. There was improved appetite in three patients, and decreased blood pressure was noted in twelve patients.

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LIBERATION OF ENDOGENOUS HISTAMINE IN MAN

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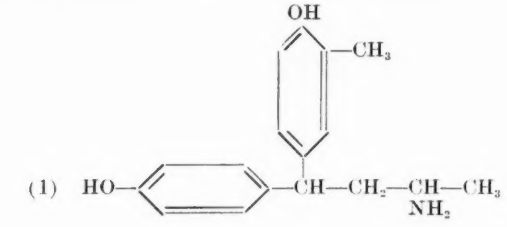
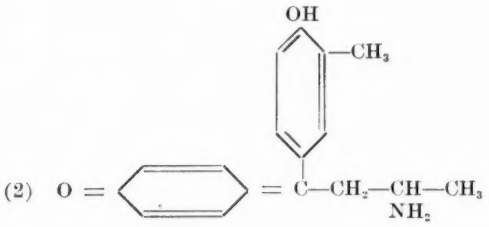
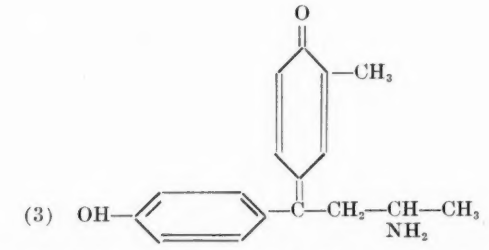
MANY chemically simple substances, when injected into an animal, release histamine from the tissues without apparent histologic damage. Those facts were recently discussed in London at the Ciba Symposium on Histamine in 1955.[1]

In man, the general symptoms provoked by the intravenous administration of histamine liberators are unperfectly known.

An opportunity for studying them systematically was provided us when clinical tests were undertaken on meriquinone of 4(4′oxyphenyl)-4(3″methyl-4″oxyphenyl) butylamine 2 and of 4′4 dihydro-4(4′oxyphényl)-4(3″methyl-4″oxyphenyl) butylamine 2 (L 1935), a depressor substance which proved to be a potent histamine liberator.







L 1935 is an equimolecular mixture of (1) with a tautomeric mixture of (2) and (3).

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Received for publication July 27, 1956.

This histamine-liberating activity, suggested in the animal by Charlier[2] and Charlier, Dallemagne, and Philippot,[3] was confirmed by Feldberg and Lecomte.[4] When injected into the hindquarters of a rat, L 1935 releases an amount of histamine equivalent to that liberated by a dose of compound 48/80 ten times inferior.

The histamine-liberating activity of this latter substance is the highest known at the present time.

METHODS

Intravenous injections of L 1935 are given in the following way: The patient, in a recumbent position, is given an infusion of an isotonic solution of glucose or sodium chloride. After the radial pulse and arterial pressure are recorded with a sphygmomanometer, L 1935 is injected through the inlet tube. Alterations in arterial pressure, pulse, skin color, and general condition of the patient are recorded.

Leukocytes are counted on a Thoma plate, after hemolysis of red blood cells by acetic acid. The platelets also are counted after dilution of the blood with Bounameaux's fluid.[5]

Gastric acidity is measured by dipping Merck indicator paper into the fluid aspirated from the patient's stomach after one day's fasting.

MacIntyre's method, as modified by Van den Driessche,[6] was applied for measuring the variations in histaminemia after a general injection of the substituted butylamine.

Normal plasma histamine, according to the above mentioned technique, fluctuates between 10 and 20 meg. per liter. No amounts higher than 25 meg. per liter were measured in normal conditions.

Sixty intravenous injections of L 1935 were given to forty patients, none of whom suffered from an allergic disease.

RESULTS

1. The intradermal injection of 100 meg. of L 1935 dissolved in 0.1 ml. of saline provokes a sudden and sharp pain at the place of injection. This extremely unpleasant sensation vanishes after three to five minutes. It gives place to itching, widely spread around the place of injection, where some erythema is also observed. This erythema spreads by successive leaps for ten minutes before settling; simultaneously, the local temperature increases by 0.5 to 1° C. The central part of the erythematous region swells progressively into an irregular papule extending peripherally during the first fifteen minutes. The swollen area itches less than its reddened circumference.

This immediate reaction decreases after thirty minutes. The affected skin remains sore and tender to pressure for another twenty-four hours.

A slight purple pigmented spot will remain for several months at the site of injection.

In a given subject, the diameters of the papule and erythema provoked by the intradermal injection will vary according to the dose of L 1935 in a constant volume (0.1 ml.) injected into the skin.

The threshold lies between 0.5 and 2 mcg. This dose, when injected, will still provoke pain and a slight itching erythema will develop after a latency of five to six minutes. The edema is slower in developing and much less obvious.

2. In order to systematize results, the manifestations observed after a *rapid intravenous injection of a single dose* of L 1935 will be recorded under four separate headings:

(a) *Slight reaction (0.1 mg. per kilogram):* Two or three minutes after the injection, the patient feels a definite impression of heat in the head. There is an unpleasant pricking on the skin of the face. Both face and neck are erythematous. Pulse rate and arterial pressure are unchanged. After five to eight minutes, the skin has recovered its normal color.

(b) *Medium reaction (0.3 mg. per kilogram):* Thirty to forty seconds after the injection, pricking begins on the skin of the face, and itching soon follows. The patient has also an impression of heat in the head. Itching then spreads to the forehead, palms of the hands, and upper part of the chest. After one or two minutes, the skin of the face is suffused. Vasodilatation then extends to the shoulders and, finally, the sides. The pulse beats faster and is easily depressible; there is a fall of 20 to 40 mm. Hg in arterial pressure, systolic and diastolic. Pulsatile headaches are sometimes observed. There is a definite impression of distress.

Erythema and the fall in blood pressure last for about fifteen minutes; sometimes the pressure will rise to a slight peak (2 to 3 cm. Hg) above normal before returning to its original value.

(c) *Serious reaction (0.5 mg. per kilogram):* Scarcely one minute after the injection, pricking in the face and itching begin, together with intense cephalic heat. Tears come to the patient's eyes; facial erythema is immediate and it soon spreads to the upper part of the chest, the abdomen, and the groin. Itching, at first limited to the face, palms, and soles, spreads to the whole body, and sometimes grows into a real sensation of ill-defined, unlocated pain. The pulse is fast, and there is a sharp fall in blood pressure. Vomiting occurs, together with a feeling of imminent defecation and an agonizing bradypnea.

After ten minutes, the reaction evolves: itching, pain, and erythema decrease; urticaria papules of various sizes then appear and spread from the neck to the shoulders and arms. The face is swollen, and edema reaches the eyelids; the upper part of the chest and the thighs display the same wheals, which finally reach the abdomen.

At last, from the tenth minute onward, there is a marked regression of cardiovascular symptoms; erythema vanishes first, then urticaria, and edema last. The patient feels, for an hour more, an unusual flare. No anxiety or late reaction follows.

(d) *Severe reaction:* This occurred with two patients. In one case, itching began as soon as the injection had been given. The face reddened but the cutaneous coloring was cut short. There was acceleration of the pulse, as well as a sudden fall in blood pressure with intense dyspnea. The patient felt sick and was pale, cyanosed, and anguished. In such reactions, Sympatol (60 mg.) is then administered intravenously. Immediately the pulse is more easily felt. The arterial pressure rises and, simultaneously, a rapidly spreading facial erythema reappears. Urticaria is observed soon afterward. The nausea subsides, and the breathing becomes more regular. The patient is quieted. No late reaction interferes with the recovery.

Another type of severe reaction occurred in an emphysematous patient, suffering from chronic asthmatic bronchitis. A severe inspiratory dyspnea set in, which lowered the rate of breathing from 18 to 10 per minute; this brought about an ashy cyanosis immediately after the fall in blood pressure. The intravenous injection of 25 mg. of Phenergan promptly relieved the patient of his bronchial spasm.

Out of the twenty-seven patients tested in this way, five showed a slight reaction and nine a medium reaction, whereas eleven showed a serious reaction and two a severe one, after the rapid injection of a *single* dose of L 1935.

3. *The slow intravenous perfusion* of a solution of isotonic sodium chloride containing *20 to 30 mg. of L 1935* for thirty to forty-five minutes entails no pathologic manifestation of any kind: erythema, fall in blood pressure, or itching. There is sometimes a slight rise in arterial pressure (10 to 20 mm. Hg).

This slow method of administration modifies neither the vascular reactions brought about by the rapid injection of L 1935 within the five minutes following the infusion nor the effects on blood pressure of histamine itself injected intravenously (150 mcg.).

4. Rapid *successive injections* of a *similar dose* (0.3 mg. per kilogram) of L 1935 (at intervals of sixty minutes) cause the progressive disappearance of above mentioned symptoms.

The reaction observed after the first injection is known. After the second administration, itching and erythema become less intense, as does the fall in arterial pressure. There is a longer latency before urticaria develops and wheals are rarely confluent. If a third injection is given, itching and erythema will still occur, but there will be no sign of a fall in blood pressure, and urticaria will not develop.

A fourth injection will provoke only a slight erythema, limited to the face.

No reaction will follow the fifth injection. From then onward, besides, the dose of L 1935 may be increased up to 0.5 mg. per kilogram without any abnormal reaction occurring.

This refractory state will still be observed on the following day: patients who have borne without any incident the successive reinjections of a similar dose of L 1935 or who showed a serious general reaction at the first contact with the substance are no longer affected in the same way if a second injection is given twenty-four hours later. A higher dose is always necessary to reproduce either the same fall in blood pressure or an edema of similar degree.

If the general phenomena are to reappear in their original intensity, a lapse of four to five days is necessary before injections are resumed.

5. We shall now proceed to analyze each of those symptoms separately:

(a) *Pricking* is the first sensation perceived by the patient. It is distinct from the immediate pain developing along the vein used for the injection. The pricking is transient; it never develops beyond the upper part of the chest and is quite different from the itching which may accompany it later. The intensity of the pricking varies according to the injected dose. It may occur without any erythema appearing and remain the only manifestation provoked by the administration of L 1935.

(b) *Itching* appears soon after the pricking, which it may often supersede. Its location is at first limited to the face and neck but, if the injected dose is increased, it spreads toward the chest, stomach, and abdomen and finally involves the whole surface of the skin. It can be very intense and bring about frantic scratching, but the areas involved are so wide that the patient is often reduced to turning about on his bed. Itching appears before the erythema and is followed, after three to five minutes, by urticaria. It disappears before this latter manifestation does. The urticaria, however, may be observed to rise without the itching being peculiarly severe. Inversely, the itching is not necessarily followed by erythema or the rising of urticarial wheals. On some parts of the skin, itching is the only evidence of the action of L 1935.

(c) *Vasodilatation* is located on the skin according to the topography described in the case of histamine infusions; it begins at once at the head and extends progressively to the skin of the neck and chest and then to the abdomen and upper thighs, but leaves the distal portion of the lower limbs unaffected. The erythema is always most intense on the nape and forehead.

(d) The proportion between the *fall in blood pressure* and the administered dose varies widely according to patients. This individual variability is quite noticeable (Fig. 1). It does not seem to depend on age or sex, but it certainly depends on the basic systolic level of blood pressure; patients with a high blood pressure are more likely than a normal subject to show an important depression.

The return to a normal blood pressure is fast. Sometimes, the systolic arterial pressure will exceed by 20 to 30 mm. Hg the original level. This particular occurrence may exceptionally develop after a brief stage of depression, and in those cases it amounts to 50 to 60 mm. Hg. At all events, the return to normal standards precedes the disappearance of erythema and urticaria.

(e) *Headaches* are of the pulsatile type. No localization in the skull is to be detected. The headaches are often unbearable, and coincide with the rise in arterial pressure rather than with the phase of depression.[7]

(f) The clinical features of the *urticaria* often vary locally. It begins on the upper part of the chest in the form of peripilary elevations, sprinkled on the erythema.

Those elevations eventually gather into small wheals, the extent of which keeps increasing until it reaches remarkable sizes; there may be spots of 10 to 15 cm^2 on the shoulder.

The fusion of those wheals induces, on the face, a hard edema which fills the supra- and subpalpebral depressions and swells lips and forehead. All those features are definitely similar to those of Quincke's edema.

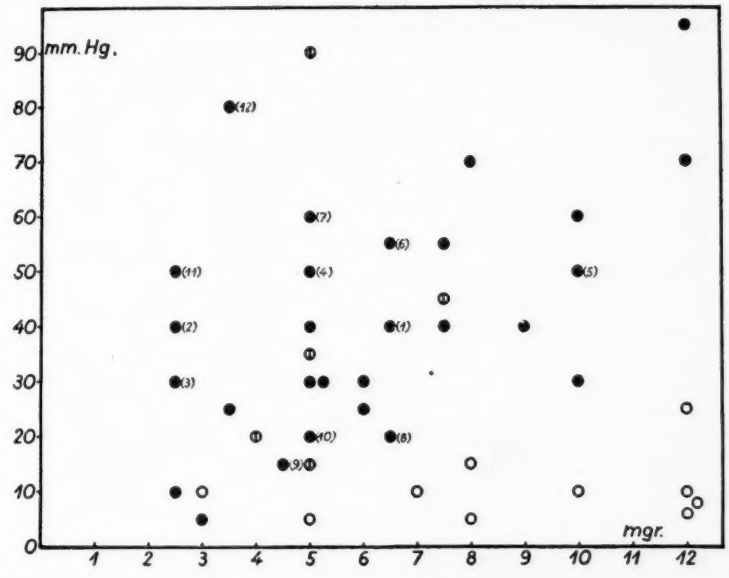

Fig. 1.—Hypotension after injection of L 1935 in normal patients or in patients previously treated with antihistaminic drugs.

In ordinates: Absolute decrease in systolic blood pressure.

In abscissas: Doses of L 1935.

The black dots represent variations elicited in normal patients; the white dots represents variations after previous treatment with procaine (200 mg.) or promethazine (Phenergan) (50 mg.). Numbers in brackets refer to Table II.

The urticaria then extends to the upper limbs, the flexor aspects of which alone are affected, then to the upper part of the back and abdomen. Finally, it covers the front of the thighs, and rarely spreads beyond the knee.

(g) *Bradypnea* developed into expiratory dyspnea in only one single case, where it grew to alarming proportions. In most cases, it is revealed by an increase in expiratory effort, with a definite feeling of retrosternal oppression. No bronchial or tracheal râles occur.

(h) *Digestive disturbances* are infrequent; nausea, vomiting, urge to defecation, and colic have been observed when high doses were injected. If a gastric catheter is introduced into the fasting patient's stomach after an intravenous injection of L 1935, the following modifications of the gastric juice will be observed: while the pH of control samples are regularly close to neutrality, the juice becomes strongly acid in general within fifteen minutes following the injection of L 1935. Table I shows details of these tests.

TABLE I

PATIENT	DOSE OF L 1935 (MG.)	DURATION OF TEST AFTER CONTROL SOUNDING (MINUTES)	pH	
			BEFORE	AFTER
G. M.	2.5	15	6	1
T. M.	5.0	30	7	5
L. G.	2.5	15	5	1
T. M.	10.0	15	6	1

(i) *The white blood cells and platelets* were counted before and at the peak of cardiovascular reactions produced by L 1935 rapidly injected intravenously in doses of 5 to 10 mg.

Together with the appearance of urticaria and cutaneous edema, a constant increase in thrombocytes and leukocytosis is observed. No accompanying variation of leukocyte differential has been noted. Neither is there any tendency of platelets to agglutination.

6. Those reactions vary under the influence of some pharmacodynamic agents.

(a) The slow perfusion of procaine (0.5 per cent) in isotonic saline up to a total dose of 500 mg. brings about no pathologic effects. The rapid intravenous injection of L 1935 then provokes the same general reactions as when no procaine has been administered (tests on six patients). Only the itching seems to be slighter. Erythema, fall in blood pressure, and pulsatile headaches are present, with the same intensity.

(b) Preliminary administration of *synthetic antihistamine* drugs alters the general reactions following upon rapid intravenous injection of L 1935 (Neo-Antergan, 50 mg.; Phenergan, 25 mg.). The rate of fall in blood pressure observed under those conditions is represented on Fig. 1. Although the dose of L 1935 is high, the induced fall in blood pressure is slight. The erythema, on the other hand, is clear cut. No other cutaneous, bronchial, or digestive disturbances can be recorded, even after a rapid injection of doses rising progessively to 20 to 30 mg.

7. Twenty milliliters of heparinized blood was taken from the cubital veins of twelve patients who were to be given an injection of L 1935. At the peak of arterial depression, a similar sample was collected.

The measuring of *free histamine in the plasma* gave the figures recorded in Table II. The rate of fall in blood pressure at the moment when the sample was taken is noted simultaneously.

Plasma histamine reaches an average of twice the normal standards when the effects of L 1935 on blood pressure are at their highest. In some patients, however, there is no increase in plasma histamine.

No strict correlation can thus be established between the fall in arterial pressure and the increase in histamine.

Fifteen minutes after the administration of L 1935, plasma histamine has come back to normal.

TABLE II. PLASMA HISTAMINE (MICROGRAMS PER LITER, AS BASE)

	BEFORE INJECTION	FALL OF BLOOD PRESSURE (MM. Hg)	AFTER INJECTION OF L 1935
1	16	40	40
2	12	40	20
3	14	30	25
4	14	50	40
5	16	50	25
6	12	55	12
7	10	60	30
8	12	20	18
9	Not measurable <5	15	12
10	Not measurable <5	20	10
11	Not measurable <5	50	12
12	Not measurable <5	80	Not measurable <5
	Mean: 13		Mean: 26

This increase is statistically significant.

In the following hour, no increase in the urinary excretion of free histamine has been noted in ten patients who had been strongly affected by the intravenous injection of L 1935.

DISCUSSION

1. At the peak of depression provoked by L 1935, the plasma contains a substance which, after extraction and concentration on succinic cotton, is observed to contract a guinea pig's ileum.

Its contracting effects persist after addition of atropine. They are strikingly sensitive to synthetic antihistamine drugs. Such effects are those of histamine.

The reactions provoked by the injection of L 1935 are to be compared to the pharmacodynamic effects of histamine. Both bring about vasodilatation, cephalic at first and thoracic later on, with a fall in arterial pressure, tachycardia, and later headache. Both provoke a strong acidity of the gastric juice in the fasting patient. Both increase the cell volume of the blood and the absolute number of platelets and leukocytes.[8, 9]

Synthetic antihistamine drugs administered previous to the intravenous injection of L 1935 check the fall in blood pressure and prevent the appearance of edema. On the other hand, they do not prevent the occurrence of facial erythema.

The interpretation of all these phenomena can lie only in the release of endogenous histamine.

The increase in plasma histamine concomitant with the fall in blood pressure induced by L 1935 is the reflection of this histamine liberation from the tissues. Only the rapid intravenous injection of L 1935 can provoke it. It is characterized by the appearance, after a short latency, of dramatic and sudden symptoms. Their intensity increases with the sensitivity to histamine of the affected apparatus. They disappear when successive injections are repeated, as the available stocks of histamine in the tissues are depleted. They are neutralized by a previous injection of antihistamine drugs.

The general reactions attesting to the liberation of endogenous histamine in man are the more complex as the quantity of released histamine increases. They are characterized by: (a) an initial stage of tegumentary vasodilatation, cephalic at first and extending later to the upper part of the chest, abdomen, and base of limbs, which rapidly induces a marked fall in arterial pressure, sometimes to the point of collapse: (b) a later increase in vascular permeability accounting for urticaria and facial edema; (c) hyperperistalsis of the digestive tract; (d) bronchial constriction; and (e) the regular occurrence of itching. Such are the symptoms of the nitritoid crisis.

What processes can, in human pathology, go together with such clinical description?

1. Nolf[10] advised intravenous injection of *peptone* in the treatment of some severe infectious conditions (for example, typhoid fever). Some patients are sensitized after several injections and show then, if later injections are given, a typical anaphylactic shock. Others display at once, soon after the first injection, flush, itching, fall in blood pressure, tachycardia, and urticaria.[10, 11]

The same clinical pattern, although of a moderate nature, is sometimes observed after the intravenous injection of d-*tubocurarine*.[12, 13]

Hypersensitivity to *cold*, which some erroneously call "physical allergy," brings about cephalic vasodilatation, fall in blood pressure, local urticaria, pyrosis, and vomiting in severe cases of prolonged exposure to low temperatures.

Evidence can then be obtained of an increase in plasma histamine and a constant change of gastric pH to acidity.[14-18]

The same clinical data, the same gastric hyperacidity, and the same increase in histaminemia are encountered in the case of *exertion urticaria*.[19]

The injection of Gonacrine (Trypaflavine) given some twenty years previously to patients suffering from gonococcus infection or severe septicemia may cause brutal vasomotor disturbances after the first injection. Their intensity and evolution may be compared to those described above; it is a crisis of cephalic vasodilatation with flutterings, anguish, and dizziness.[20]

Analogous vasomotor reactions are described in the course of *arsenotherapy* without any previous contact with the drug necessarily justifying the frequently quoted anaphylactic pathogenesis.[21]

In short, the reaction that was willingly induced in man by the injection of L 1935 is similar to the one that sometimes follows on the rapid intravenous injection of *histamine-releasing substances, authenticated in animals:* peptone, curare, Trypaflavine, organic arsenical compounds, morphine, atropine, bile salts, and polymyxins.[1]

It is a result, in some subjects, of exposure to the cold or excessive muscular exertion.

It may be among the prodromes of infectious hepatitis.

2. The symptomatology of patients suffering from anaphylactic shock also depends on the conjugated action, to various degrees, of six factors: itching, capillary-arteriolar vasodilatation responsible for the collapse, increased intestinal peristalsis, contraction of bronchial muscle fibers, mucous hypersecretion, and increased vascular permeability. It consists in an injury of smooth-muscle fibers, with a further excitation of prurigenous nervous fibers[21, 22] analogous to that one consecutive to a rapid intravenous injection of L 1935.

The administration of sympathomimetic amines may quickly amend this collapse, or remain inactive. In that case, synthetic antihistaminic drugs bring relief.

Indeed, the plasma collected during the shock period proved, in two of our patients, to have definite depressor effects due to the presence of histamine, pharmacodynamically identified. In a third patient, histaminuria, recorded during the shock period, was proved to have increased.[23]

Thus, the analogies between the symptomatology of anaphylactic shock and the effects of histamine liberators are accounted for.

CONCLUSIONS

1. Intravenous administration of L 1935 causes, from the dose of 0.1 mg. per kilogram, a complex general reaction, the more severe as the administered dose increases.

This reaction is characterized by the conjugated action of six factors: itching, cephalic vasodilatation with a fall in blood pressure, spasm of bronchial muscles, hyperperistalsis of the muscular coats of the intestine, augmented digestive secretions, and increased vascular permeability.

These reactions are peculiar to rapid intravenous injections of the substance. They become milder when successive reinjections are repeated, or when synthetic antihistamine drugs are given.

At the climax of the fall in blood pressure, plasma histamine is doubled.

2. The clinical reaction to intravenous injection of L 1935 depends on the histamine liberation from tissues provoked by this substance.

Its nature is similar to that of the classical nitritoid crisis. The latter can be generally related to the quick liberation of histamine from tissues.

3. Anaphylactic shock manifests itself in man by symptoms analogous to those provoked by the release of endogenous histamine. It seems thus suggested that anaphylactic shock is of histaminic origin.

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ALLERGY TO CADDIS FLY (TRICHOPTERA)

I. The Insect

Howard Osgood, M.D., Buffalo, N. Y.

SINCE Parlato[1, 2] reported the first case of a patient having respiratory symptoms due to allergic sensitivity to the caddis fly in 1929, many additional cases have been encountered and treated in the region of the Niagara River and much local information concerning these insects has been accumulated.

Because the caddis fly occurs throughout the United States and Canada, as well as all other parts of the world, and presumably can be an unrecognized cause of allergic symptoms, it seems appropriate to present a résumé of knowledge relative to this insect and, more specifically, to describe the circumstances of its presence around Buffalo, New York, and Fort Erie, Canada.

ZOOLOGIC CLASSIFICATIONS

In systematic zoology, the caddis fly (*Trichoptera*) is classified as follows[3]:

Phyllum:	*Arthropoda*
Class:	*Hexapoda* (insects)
Sub-class:	*Pterygota* (winged insects)
Order:	*Trichoptera* (caddis fly)

The order *Trichoptera* is divided into numerous genera and species.

The *Trichoptera* (hairy-winged) are characterized by the presence of minute hairs (actually hairlike scales) covering the wings. In this respect, they are contrasted with such other orders of insects as the *Lepidoptera* (scaly-winged) comprising moths and butterflies, and the *Hymenoptera* (membranous-winged) comprising bees, wasps, ants, etc. The May flies, which Figley[4] has shown may also cause allergic sensitivity, belong to a separate and more primitive order of *Pterygota,* namely, the *Ephemerida.*

GENERAL DESCRIPTION AND HABITS

The caddis fly is essentially an aquatic insect; all of its life cycle, except for the brief adult stage, is spent in the water. The eggs are laid and hatched in the water. The larva goes through a number of successive molts, during the earliest of which it is free swimming. Subsequently, it settles to the bottom where it builds about itself an elongated "case" consisting of small particles of stone, sand, or vegetable material held together by a silklike salivary secretion and open at the ends so that the water may flow through. It some species, the case is mobile; in others, it is attached to rocks, ledges, pebbles, or other objects under water. The food consists of microorganisms and other organic material brought to the larvae by the current of the water.

Received for publication Oct. 8, 1956.

Where bottom conditions and water flow are favorable, the beds contain uncounted numbers of larvae and these form a highly important supply of fish food.

After reaching full growth, the larva pupates and, after metamorphosis, rises to the surface of the water where the adult (imago) quickly emerges and flies to shore. There mating takes place under the cover of the leaves of trees and shrubbery. After mating, the female flies back to the water to deposit her eggs; the male remains ashore and dies. In regions where the water freezes over seasonally, the insects survive the winter in the larval or pupal form.

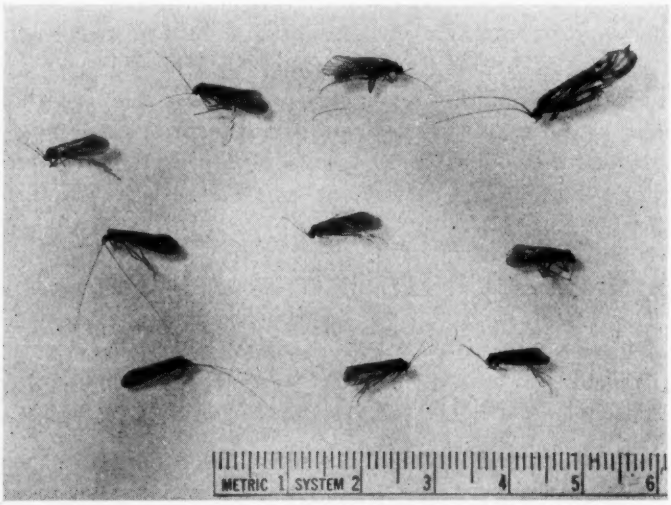

Fig. 1.—Caddis flies of the Niagara area. Several species are shown approximately life size.

The adult caddis fly varies in length from 3 mm. to 12 mm., according to the species (Fig. 1). In general color, the species vary from a light tan to a very dark brown and the wings, especially when folded, appear opaque. A few species possess some mottling of the wings but none, I believe, are brightly colored, as are moths and butterflies.

Two pairs of wings spring from the thorax, the anterior pair being somewhat longer than the posterior pair. When the insect is at rest, the wings are always folded backward along the sides of the body, the anterior pair covering the posterior pair and the margins meeting above the back. The folded wings thus form a steep-sided "tent" which covers the length of the body and extends somewhat beyond the tip of the abdomen posteriorly. The thorax also bears three pairs of walking legs.

A pair of antennae project forward from the head. These are finely "haired" and are at least as long as the body; in many species they are much longer. None of the caddis flies are biting insects, the mouth parts being weakly developed. The abdomen ends posteriorly without cerci (a pair of long projecting organs such as are present in the *Ephemerida*).

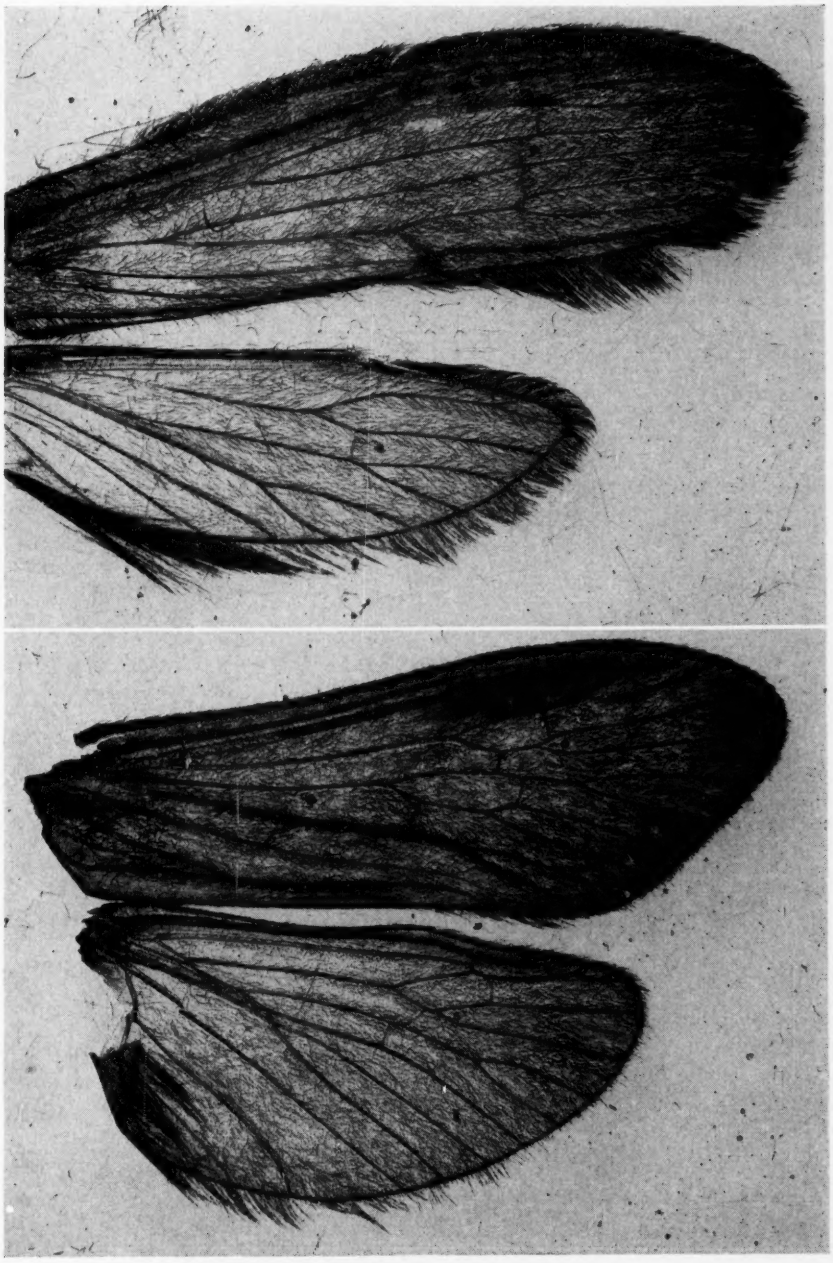

Fig. 2.—Caddis flies of the Niagara area. Fore and hind wings of two species. Note the slight differences in hairiness on the surfaces and the large difference along the margins. Taxonomically, one criterion for the identification of species is the venation of the wings.

The hairs are present over the surfaces of both pairs of wings, most thickly on the anterior pair. (Figs. 2 and 3.) Around the wing tips and along the posterior wing margins, there is usually a fringe of longer hairs. All these hairs are easily dislodged. They and the particles of the dead and dried bodies float in the air and are the means by which the symptoms are induced when inhaled by a patient allergic to them.

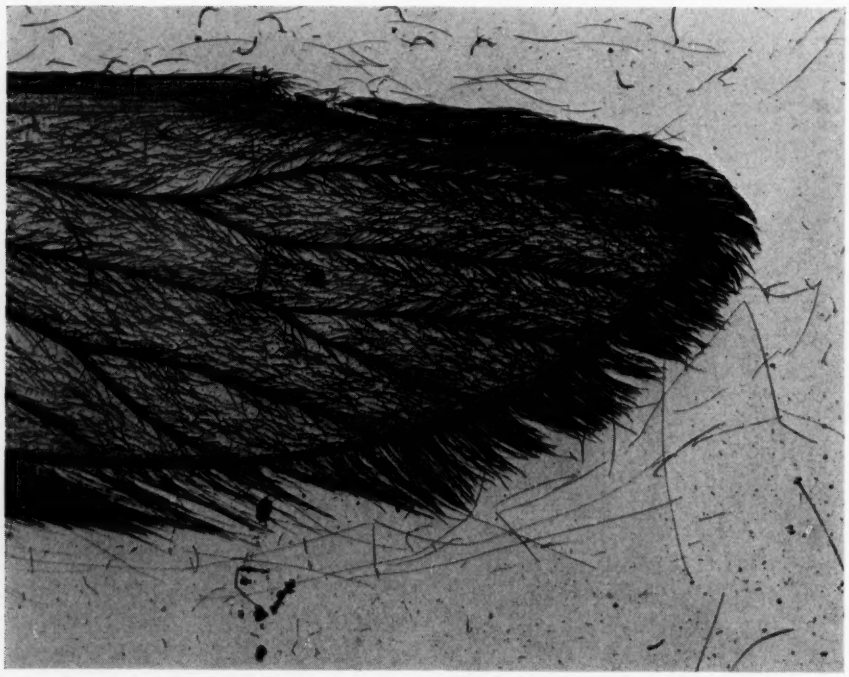

Fig. 3.—Caddis flies of the Niagara area. A wing tip further enlarged. Note the dislodged hairs lying free.

When alighted on a solid surface, the caddis fly is active, often being a nuisance to man by crawling into the hair and over the skin, into sleeves, and down the neck.

The flight is not strong, although the females can fly against a moderate breeze to reach the water for egg laying.

ENVIRONMENTAL CONDITIONS IN THE NIAGARA AREA

Lake Erie lies in a generally east-west direction; it is the shallowest, and hence the warmest, of the Great Lakes. Its outlet at the eastern end forms the head of the Niagara River, which flows for thirty miles in a northerly direction to empty into Lake Ontario. The famous Niagara Falls and the turbulent rapids are in the middle third of its length. The cities of Buffalo and Fort Erie lie on either side of the head and upper reaches of the river.

The bottom of the lake outlet and of the first two or three miles of the river consists of flat rock ledges partly covered with rocks and gravel. Where

the lake narrows, there is a slow current in the water, which gains speed as it nears the river, until it is eight to ten miles per hour between the two cities, at which point the river is about one-half mile wide. The current and the character of the bottom in this area create ideal conditions for the caddis fly larvae. Two to three miles downsteam, where the river widens and divides into two broad branches, the bottom changes and the larval beds greatly decrease. It is in the vicinity of the two cities, therefore, that the caddis fly occurs in great abundance (Fig. 4).

On the Fort Erie side of the river the land does not rise far above the water level; dwellings and stores extend practically to the water's edge. In Buffalo the land rises considerably higher and the river front is occupied largely by harbor, canal, parks, and industrial plants; the dwellings stand farther back from the river's edge.

Due to these features, the caddis fly nuisance is more serious on the Fort Erie side. There the doors, windows, and porches within several hundreds of yards of the river must be tightly screened. For decades, it has been impossible to do house painting in the summer or to sit out-of-doors in comfort. Cases of allergy to the caddis fly are more frequent in the Fort Erie population because of the more intense exposure.

SURVEY OF LOCAL SPECIES

During July and August, 1950, on the initiative and with the support of the Lions Club of Fort Erie, a detailed survey was undertaken of the local occurrence and habits of the caddis fly. This was carried out with the advice and active participation of representatives from the Departments of Zoology and Entomology of the University of Toronto and from the Division of Entomology of the Canadian Government in Ottawa. From the reports of this survey,[5, 6] I have taken most of the material in the following two paragraphs. No similar survey has been made on the United States side. Fig. 4 shows the breeding beds and the approximate land area of infestation on the Canadian side.

Twenty-three species of caddis fly were identified by the Canadian survey. Of the thousands of individual larvae and adults collected, the bulk were of the genus *Hydropsychida* and 75 per cent were of the species *Hydropsyche bifida* Walker. An additional 25 per cent consisted of the following species: *Oecetis avara* Banks, *Hydropsyche placoda* Betten, *Neureclipsis crepuscularis* Walker, and *Macronema zebrata* Hagen. All are characteristic of rapid streams.

The Canadian investigators found that the adults emerge from the water at a fairly uniform rate throughout the twenty-four hours of the day. They fly ashore and infest the foliage for a distance of many hundreds of yards inland. They may remain in the bushes and trees for several days.

In the late afternoon and early evening, the females, after having mated, begin a general flight over the land and then out over the head of the river and lake outlet, where they deposit their eggs by touching the surface of the water with the tip of the abdomen.

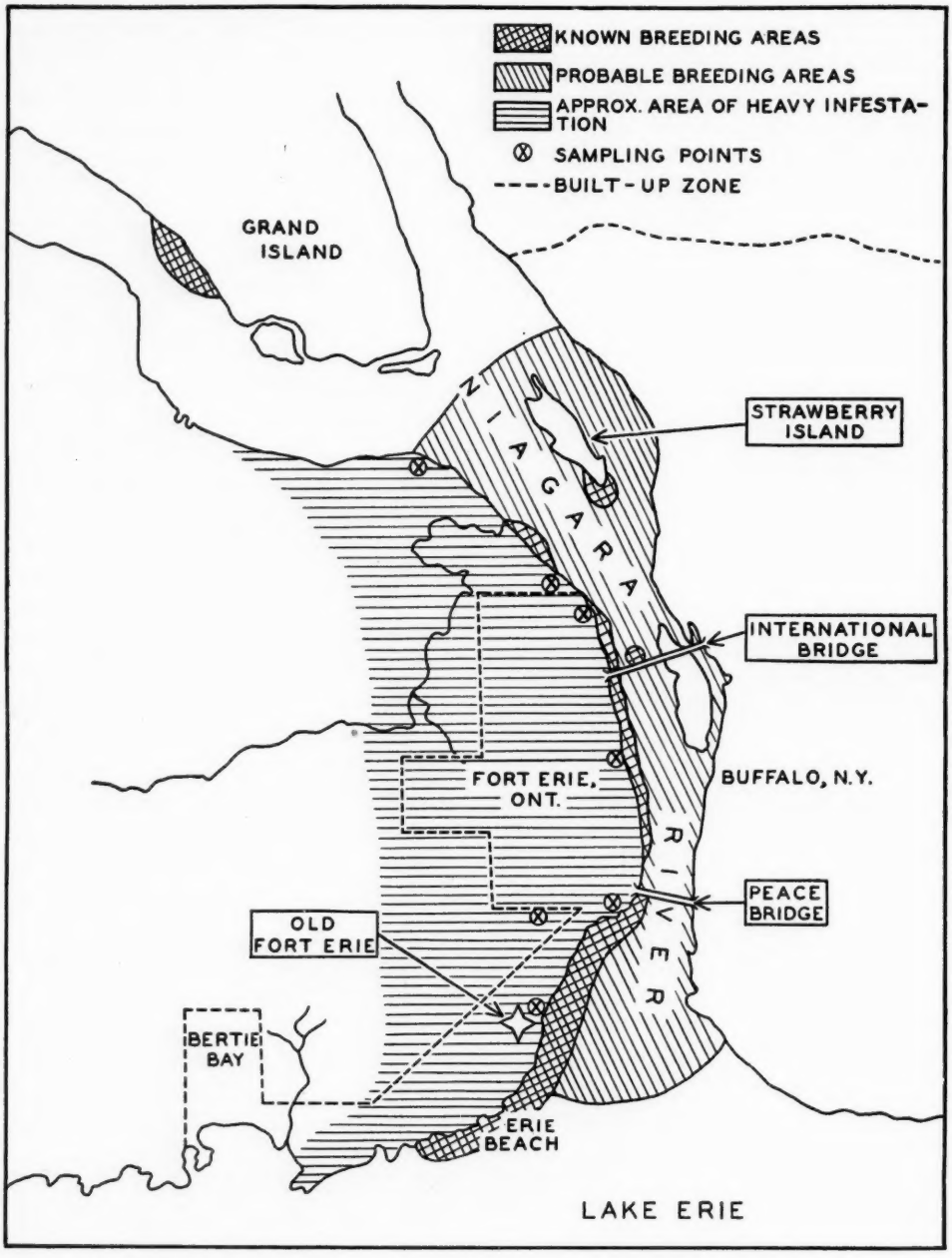

Fig. 4.—Map of Niagara area concerned. Shown are the known and probable underwater breeding areas and the land area of infestation in Fort Erie, Canada. (From the report by D. G. Peterson in *The Canadian Entomologist*, April, 1952. Courtesy of Canada Department of Agriculture.)

CADDIS FLY SEASON

Since my attention was first directed to the caddis fly in 1929 by Parlato's report, I have occasionally seen caddis flies in the air, singly or in small numbers, as early as June 4 and as late as October 1. However, the actual flight season in this area begins about mid-June and lasts until the end of September. During this period the insects may be seen flying daily, and numbers are always present in the foliage adjacent to the river.

FLIGHT DENSITY AND FACTORS INFLUENCING IT

The flight density of the caddis flies in the Niagara area varies considerably during the season, due to factors to be discussed. Several times at irregular intervals during the season, the caddis flies appear in great swarms which may persist for several consecutive days. At such times they form dense banners of hovering insects, several feet long, to the lee of tree tops, bushes, and gable-peaks. They swarm around lights and enter any uncovered house openings. When the late afternoon flight of the females toward the water is in progress, they may fill the air as snow in a storm.

One swarm toward the end of June, 1950, extended for nearly two miles along the river border and inland for approximately one-half mile and to a height of about 30 feet. I estimated an average of five caddis flies per cubic foot of air. A little arithmetic will show that this one swarm may have contained over 2 billion insects. During another swarm on July 28, 1950, I counted about 5,000 caddis flies resting on the glass of a lighted window, measuring 6 by 7½ feet, at the Canadian Immigration Office 200 yards from the river edge. The last "swarm" in 1950 occurred on August 21, but fairly dense flights occurred intermittently until September 1 and then decreased until the end of September (Fig. 5).

Although these recurring swarms are a rather striking feature, it should be emphasized again that the flies are present along the borders of the river in appreciable numbers throughout the summer and so constitute a continuing source of wing hairs and body particles in the air.

In order to determine, if possible, the factors influencing the initial appearance of the adult caddis flies in June and the subsequent variable density of the insects, I made observations during the months of May through September, 1950, on both sides of the river, noting grossly the numbers of caddis flies on wing. Later I secured from the Buffalo Division of Water their records of the daily temperature of the water taken at the outlet of Lake Erie during the same months of 1950. I also secured from the United States Weather Bureau at Buffalo their complete meteorologic data for the same period. Correlating the three sets of data, I can report the following (Fig. 5):

As the lake (and hence the river) warmed up from the winter low, the critical water temperature leading to the first appearance, on June 5, 1950, of the adult caddis flies was about 60° F., their appearance in large numbers occurred at 62° F. on June 15. The first "swarm" occurred on June 24, when the water temperature had been 63° F. for four days. The water remained

APPROXIMATE FLIGHT DENSITY IN AIR OF CADDIS FLIES IN 1950

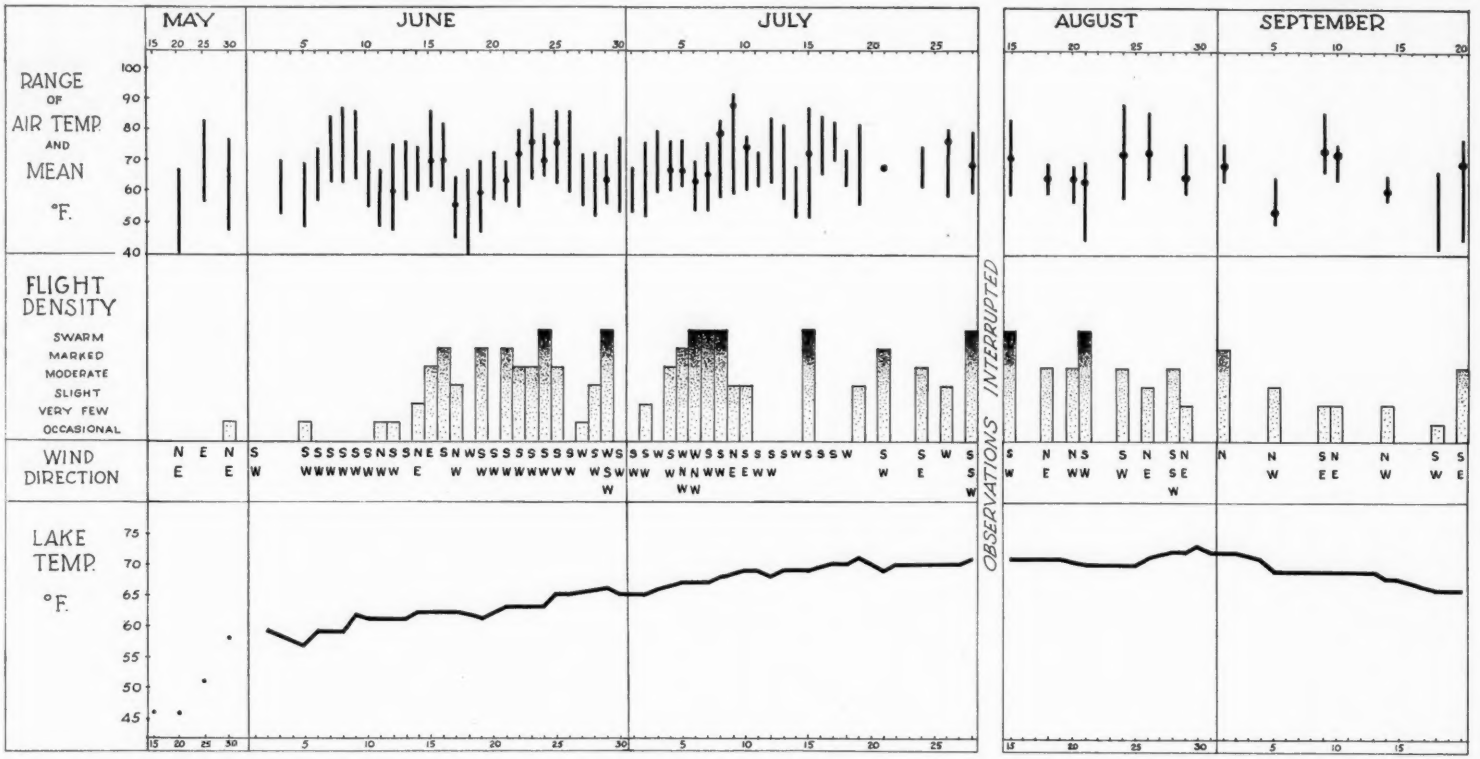

Fig. 5.—The shaded columns represent the density of the flies in the air at the time of observation. Blank spaces indicate days on which no observations were made.

above 65° F. from June 25 until September 16, during which period eight additional "swarms" occurred. A maximum water temperature of 72° F. was recorded from August 28 to September 2, during which period no "swarms" occurred, although the flies were present in large numbers. Above 65° F., minor fluctuations of 2 to 3 degrees of water temperature appeared to have no relation to the variations in flight density or to the occurrence of "swarms."

As to meteorologic conditions, no appreciable numbers of caddis flies were on wing when the air temperature was below 60° F. No marked flight density and no swarms were noted unless the air temperature was above 63° F. The summer fluctuations of air temperature between 63° F. and 90° F. appeared to have no direct correlations with high or low flight density, although most of the swarms occurred when the temperature was above 64° F. Fight density was definitely at a minimum in the presence of a strong breeze and of rain. It was favored by quiet air. It probably is also influenced by the maturation at different times of successive batches of larvae of the same age.

The adult caddis flies do not wander far from the lake outlet or the river. Ordinarily they are most abundant along the immediate borders of the water and inland for a distance of 300 or 400 yards. In the presence of a swarm, or a fairly strong cross breeze, they may occur in small numbers as far as one and one-half to two miles from the river. A patient sensitive to the emanations of the caddis fly may secure practically complete freedom from symptoms at a distance of five miles from the river.*

COMMENTS

The region of the outlet of Lake Erie and the upper Niagara River presents environmental and physical conditions conducive to the development in large numbers of several species of caddis fly indigenous to flowing water. These conditions include a water bottom of many hundreds of acres in extent favorable to the growth of the larvae, a season of nearly four months during which the water temperature permits the maturation of these larvae, an abundant food supply derived from a relatively warm lake, ample foliage near the water in which mating can take place, and meteorologic conditions (generally gentle air movements, mild air temperature, and no continuous rain) which permit easy flight of the insects. In a large human population living adjacent to the breeding areas many persons are exposed to the emanations of the flies; this leads to the occurrence of allergic sensitivity to them in susceptible persons.

Elsewhere, caddis flies occur in small numbers throughout the countryside along innumerable streams, large and small, favorable to their growth; some species breed in ponds and lakes. There is, therefore, the possibility of widespread but light exposure to their emanations and, theoretically at least, of the occasional appearance of allergic sensitivity to them in the local human population. I have no information as to what flight density (intensity

*Hairs identical morphologically to those from the caddis fly have been observed on slides exposed for pollen counts on the roof of a ten-story hospital building in Buffalo, situated about one and seven-eights miles from the bank of the Niagara River. Actual "hair counts" have not been done due to the difficulty in accurately differentiating caddis fly hairs from other insect hairs and from some plant hairs present on the slides.

of exposure) is necessary for the appearance of such sensitivity. It probably does not need to be nearly as great as that existing in the Niagara area. Of much help in answering this question would be the identification of sites in the United States and Canada where caddis flies are numerous and in close proximity to populated areas. If any reader of the present article can give me information about such sites, I would be most grateful.

Populated areas, of which I am presently aware, where caddis flies are numerous, are: Cornwall, Ontario, and Lachine, Quebec, on the St. Lawrence River; Ottawa on the Ottawa River; the Delaware Water Gap near Stroudsburg, Pennsylvania; Ogdensburg, New York; and some areas in California.

Parlato, La Duca, and Durham,[8] in 1934, reported that the caddis flies are found locally in the Adirondack and Catskill mountains and around the Finger Lakes and Chautauqua Lake in New York State, and also in Toledo, Cleveland, Chicago, North Chicago, Milwaukee, Madison (Wisconsin), Port Arthur (Ontario), and Minnesota.

Regarding the universal distribution of the order of *Trichoptera*, it is represented by numerous varieties on every continent of the world,[7] in Japan, in the East and West Indies, in Australia and New Zealand, in Greenland.

A second article is in preparation covering the clinical aspects of caddis fly allergy. Suffice it to say here that cases of this sensitivity are sufficiently common in Buffalo and Fort Erie that allergic patients are tested routinely with caddis fly extract along with the usual environmental inhalants. The symptoms (allergic rhinitis, conjunctival irritation, and asthma) produced by the caddis fly allergy frequently require hyposensitizing injections of the caddis fly extract and the results are comparable with similar treatment for pollinosis.

SUMMARY

The physical and environmental features existing at the outlet of Lake Erie and at the head of the Niagara River create conditions under which caddis flies develop in great numbers. The immediate proximity to these waters of two cities—Buffalo and Fort Erie—exposes a large population to the emanations of these flies and results in the not uncommon occurrence of allergic sensitivity to them.

A recent survey of the area disclosed the presence of twenty-three different species of caddis fly. Ninety-five per cent of the thousands of specimens collected, however, belonged to five species.

The habits of the local species are described and observations are reported on water and meteorologic factors which influence the seasonal occurrence and the flight density of the adult flies and hence the intensity of human exposure to them.

A brief note is made on the world-wide distribution of the caddis fly, and on other areas in the United States and Canada where concentrations of them exist.

A second article is in preparation, covering the clinical aspects of allergy to the caddis fly.

I wish to acknowledge with gratitude my indebtedness to Mr. A. H. Thompson, president of the Fort Erie Lions Club, for keeping me informed as to the progress and results of the Canadian survey of the flies; to Mr. Henry F. Wagner, chemist in charge of the Filtration Plant, Buffalo Division of Water, for his cooperation in the study of caddis fly larvae in the filter beds and for making available his records of water temperature, etc.; to Dr. R. O. Rilett, professor of entomology, University of Buffalo, for advice during the survey and for reviewing this paper; to the Division of Entomology, Dominion Department of Agriculture, Ottawa, for permission to use their published map of the area concerned; to the Buffalo Museum of Natural Sciences for the loan of their slide mounts of caddis fly wings; to Mr. Melford D. Diedrick, medical illustrator, and the Audio-Visual Center, University of Buffalo, for preparing the illustrations and chart.

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RAGWEED POLLEN DESENSITIZATION IN HAY FEVER

Relationship to Skin Test Reaction, Dosage, and Clinical Results

Louis Tuft, M.D., and V. Muriel Heck, M.T., Philadelphia, Pa.

IN A previous publication,[1] we discussed the question of the effect of specific pollen densensitization upon the skin test reaction. The responses to our questionnaire survey revealed a definite difference of opinion among practicing allergists concerning this point, similar to that contained in allergy textbooks[2] and in the literature. For example, most of those who responded felt that there was a distinct lessening of the skin test reaction; in fact, a few stated that they utilized the conversion of the reaction from positive to negative as an indication of remission or "cure" and based their cessation of treatment upon this finding. They agreed, therefore, with the opinions expressed in previous publications by Colmes[3] and Vaughan,[4] who likewise believed, from the results of their investigations, that cutaneous sensitiveness decreased considerably even from a single season's treatment and that this decrease could be utilized as a criterion of subsequent dosage.

A different opinion was expressed in other responses to the questionnaire by those who felt that pollen therapy had little or no effect upon the skin test reaction and that one should not depend upon this alteration to determine the clinical effect of treatment. This opinion was in accord with that expressed in previous publications by Markow and Spain[5] and by Pearson,[6] all of whom found, from their investigations, that treatment produced little change in skin test reactions and that even when a reduction took place, it was temporary. In view of the still prevalent difference of opinion among allergists, we attempted to provide more definite data by reviewing the case records of those ragweed hay fever patients who had received treatment regularly, both by the preseasonal method and by the perennial method, for periods of one to ten years or longer and who had been retested at varying intervals. These results were included in the report of our initial retest study in which we showed that pollen therapy apparently had little effect upon the skin test reaction except in those patients treated more than five years. In the latter group, there seemed to be definite reduction in a statistically significant proportion; however, the skin test became negative in only two patients in the entire series, and in both of them the reaction was reduced from slight to negative.

From the Clinic of Allergy and Applied Immunology, Temple University Hospital and School of Medicine.

Presented at the twelfth annual meeting of The American Academy of Allergy, Feb. 6 to 8, 1956, St. Louis, Missouri.

Received for publication Jan. 25, 1956.

In our first study we were concerned solely with changes in skin test reactions observed upon retesting, and therefore reported only the data which pertained to that phase. While we were reviewing this material, however, the opportunity presented itself to obtain more definite information regarding the effect of pollen therapy upon factors other than alteration in the skin test reaction, information which might interest the practicing allergist but about which, at least as indicated by a survey of the literature, difference of opinion still exists, perhaps because of a lack of more conclusive evidence. The present report contains the results of investigations of these factors. More specifically, we attempted to determine the following:

1. The effect of pollen therapy upon (a) the skin test reaction and (b) the clinical results.

2. The effect of dosage upon (a) the skin test reaction and (b) the clinical results.

3. The effect of the duration of treatment upon (a) the skin test reaction, (b) the clinical results, and (c) the dosage.

4. The effect of the method of treatment (perennial or preseasonal).

METHOD

As already indicated, we reviewed the case records of 200 patients with ragweed hay fever (referred to later as the "combined group"); 100 of these patients had received treatment each year by the preseasonal method, and the other 100 were treated perennially. The records were obtained from the private office files of the senior author (L. T.) and were selected by going through the file, starting with the letter "A" and continuing until 100 preseasonally treated and 100 perennially treated patients were obtained.

In making our selection, we tried, whenever possible, to choose patients with pure ragweed hay fever. However, clinical experience has shown that most hay fever patients have multiple sensitivities, both cutaneous and clinical. Thus, many have associated spring hay fever (grass and/or tree), others have perennial allergic rhinitis from sensitivity to allergens other than pollens (for example, inhalants or foods); and finally, some may have pollen asthma either associated with the hay fever or as the principal manifestation. When this is true, appropriate treatment is instituted in addition to ragweed pollen desensitization. These latter considerations apply to the 200 patients selected for study—in the majority, ragweed hay fever was the major manifestation but some had associated spring hay fever and/or perennial allergic rhinitis. A few had associated seasonal asthma, although none had pure pollen asthma. However, since most of our comparisons concerned only the sensitivity to ragweed and were made in the same patients, we felt that the presence of associated sensitivities or their treatment would not interfere significantly with the results obtained or with the statistical evaluation. This also was true of any comparisons between those treated preseasonally and those treated perennially, since the associated sensitivities other than ragweed in both groups were managed in the same manner.

All patients were tested initially with increasing concentrations (0.0001, 0.001, 0.01, and 0.1 mg. N) of ragweed pollen extract (standardized by the total N method) until a marked reaction was obtained. Subsequent retests always were done with the same concentration which produced the largest reaction; thus, if R. 0.01 gave a marked reaction initially, the patient was retested with this concentration on subsequent visits. The ragweed pollen extract was prepared from a mixture of equal parts of giant and dwarf dry ragweed pollen extracted in the standard manner, according to the Coca method. Fresh extracts were prepared at least twice a year, so that the extracts used in the retests usually were fresh ones and similar in most respects to the extracts previously employed. Furthermore, the same extracts employed for the tests also were utilized in treatment.

Finally, all patients, regardless of the method of treatment (preseasonal or perennial), were given appropriate medication needed for relief of any active hay fever symptoms, but in no instance was drug therapy utilized as the sole or major form of treatment or was it sufficient to interfere with proper interpretation of the results obtained.

In addition, we were careful to obtain the following data: the duration of treatment, the skin test reaction at the time of the initial test (and especially of the final retest), the interval between these tests, the change in reaction (decrease, no change, or increase), the maximum dosage (in total N) of pollen extract that the patient was able to tolerate without inducing either a severe local or a constitutional reaction, and the clinical results of treatment (listed as poor, fair, or good). The latter was largely a subjective determination dependent upon the opinion of the patient but was supplemented by the notes contained in the patient's history record. Since all but three of the patients had been retested each year with the same concentration of pollen extract and by the same person (V. M. H.) for a period up to nine years (1945 to 1953, inclusive), we felt that any possible variation in symptoms resulting from yearly difference in pollen exposure thus would be corrected and a fair comparison assured. All the results were subjected to expert statistical analysis* under the supervision of Dr. H. Hamilton. The figures obtained were calculated and analyzed according to standard statistical methods using the chi-square formula and the law of probability (p). In recording our results, therefore, we use the term "significant" to indicate that the relationships obtained were not chance ones, whereas the figures for "p," showing the level of confidence, are included in the tables.

RESULTS

Effect of Pollen Therapy.—

Upon skin test reactions: The data obtained are similar to those already included in our first report[1] and are shown in Table I. It will be observed that a significant number (51 per cent) of the combined group showed a decreased

*The authors are grateful to Miss Eileen Beier for her statistical analysis of our data and to Dr. H. Hamilton, Professor of Psychology, Temple University, Philadelphia, Pennsylvania, for his supervision.

skin test reaction as compared with the number showing an increase (17 per cent) or even those showing no change (32 per cent). On the basis of these results, we can conclude that a decrease in skin reaction is significantly more likely to occur than an increase. This is true regardless of whether the method of treatment employed is preseasonal or perennial.

Upon the clinical results: The data obtained are also shown in Table 1. Inspection of the table (vertical column marked with †) shows that a significant number (73 per cent) of the 200 patients had good results as compared with 20.5 per cent who had fair results and only 6.5 per cent who had poor results. Similar results were obtained for both the preseasonally and the perennially treated groups, indicating that the method of treatment had little effect upon the results.

Further analysis was made concerning the relationship of the clinical results of treatment to the alteration in the skin test reaction. Analysis of our data (columns in Table I marked with *) indicates that in the combined group the percentage (40.5 per cent) of patients whose reactions decreased had good results was considerably larger than those whose reactions either remained the same (22.5 per cent) or increased (10.0 per cent). These results were the same regardless of whether the patients were treated perennially or preseasonally.

TABLE I. EFFECT OF POLLEN THERAPY UPON SKIN TEST REACTIONS AND UPON CLINICAL RESULTS

CLINICAL* RESULTS OF TREATMENT	COMBINED GROUPS				PRESEASONAL GROUP				PERENNIAL GROUP			
	SKIN TEST REACTIONS*				SKIN TEST REACTIONS				SKIN TEST REACTIONS			
	DE-CREASE	SAME	IN-CREASE	TO-TAL†	DE-CREASE	SAME	IN-CREASE	TO-TAL	DE-CREASE	SAME	IN-CREASE	TO-TAL
Good	40.5	22.5	10.0	73.0	38.0	25.0	9.0	72.0	43.0	20.0	11.0	74.0
Fair	7.0	7.5	6.0	20.5	6.0	8.0	7.0	21.0	8.0	7.0	5.0	20.0
Poor	3.5	2.0	1.0	6.5	4.0	2.0	1.0	7.0	3.0	2.0	1.0	6.0
Total‡	51.0	32.0	17.0		48.0	35.0	17.0		54.0	29.0	17.0	

*p = between <.05 and >.02 (statistically significant).
†p = <0.001 (statistically significant).
‡p = <0.001 (statistically significant).
(*Note:* All figures represent percentages.)

We may conclude, therefore, that pollen therapy in hay fever patients generally will produce good clinical results, especially in those whose skin test reactions decrease following treatment.

Effect of Dosage.—

Upon skin test reaction: Analysis of these data (Table II) showed that alteration in the skin test reaction was significantly related to dosage in the combined group. Inspection of the results obtained reveals, in the first place, that a majority (79.5 per cent) of the 200 patients were able to take pollen extract in dosages of 0.01 N or greater. In the latter group of patients, as indicated in the table, a higher proportion (43.5 per cent) of subjects with dosage of 0.01 N or over (vertical column marked with *) were more likely to have a decreased skin test reaction than either no change (24.5 per cent) or an increase (11.5 per cent), in contrast to those patients who received less

than 0.01 N in whom no similar relationship existed. This is even more apparent and significant in those patients who received a dosage of 0.1 N or over (vertical column marked with †), 11.5 per cent of whom showed a decreased skin test reaction compared with 2.5 per cent who showed no change and 0.5 per cent who had an increase.

TABLE II. EFFECT OF DOSAGE ON SKIN TEST REACTION (COMBINED GROUPS)

EFFECT ON REACTION	LESS THAN 0.01 N*	0.01 N AND OVER*	LESS THAN 0.1 N†	0.1 N AND OVER†
Decrease	7.5	43.5	39.5	11.5
Same	7.5	24.5	29.5	2.5
Increase	5.5	11.5	16.5	0.5
Total	20.5	79.5	85.5	14.5

*p = <0.05 >0.02 (statistically significant).
†p = <0.001 (statistically significant).
(*Note:* All figures represent percentages.)

These results suggest that the size of the dose reached apparently is related to the change in skin test reaction; that is, the larger the dose, the more likely is a reduction in skin test reaction to occur.

Upon clinical results: The data pertaining to the effect of dosage upon clinical results in the combined group are shown in Table III. Analysis of the

TABLE III. EFFECT OF DOSAGE ON CLINICAL RESULTS OF TREATMENT (COMBINED GROUPS)

CLINICAL RESULTS OF TREATMENT	LESS THAN 0.01 N	0.01 N AND OVER	LESS THAN 0.1 N*	0.1 N AND OVER
Good	12.5	60.5	59.5	13.5
Fair	5.5	15.0	20.0	0.5
Poor	2.5	4.0	6.0	0.5
Total	20.5	79.5	85.5	14.5

*p = <0.01 >0.001 (statistically significant).
(*Note:* All figures represent percentages.)

results obtained again reveals that pollen therapy, regardless of the dose, is more likely to bring about good results than either fair or poor results. But, in addition, the results obtained definitely show that the higher the dosage, the better are the results likely to be. Thus, as indicated in Table III, a significant percentage (13.5 per cent) of patients getting a dosage of 0.1 N or over (vertical column marked with *) had good results, compared to a total of 1.0 per cent having fair or poor results. This is a larger ratio (13.5 to 1) than patients getting a dosage of less than 0.1 N, in whom 59.5 per cent had good results compared to 26.0 per cent having fair and poor results. This would seem to indicate that the results of treatment in hay fever patients are influenced favorably by high dosages.

Effect of Duration of Treatment.—

Upon the skin test reaction: Since the length of treatment for patients in the combined group varied from one year to ten years or more, the data for different periods of time were analyzed. The results obtained are shown in Table IV. Inspection of the latter shows that the longer the patient is treated,

the more likely is the skin test reaction to decrease. This is particularly true of patients treated for one to five years, representing the majority (56 per cent), compared with those treated for more than five years (44 per cent).

TABLE IV. EFFECT OF DURATION OF TREATMENT ON SKIN TEST REACTION (COMBINED GROUPS)

EFFECT ON REACTION	1 YEAR	2 YEARS AND OVER	1 TO 5 YEARS*	6 YEARS AND OVER*	1 TO 10 YEARS	11 YEARS AND OVER
Decrease	4.0	47.0	24.5	26.5	43.5	7.5
Same	4.5	27.5	22.5	9.5	31.0	1.0
Increase	1.5	15.5	9.0	8.0	15.5	1.5
Total	10.0	90.0	56.0	44.0	90.0	10.0

*p = <0.02 >0.01 (statistically significant).
(*Note:* All figures represent percentages.)

As shown in the table (vertical columns marked with *), skin test reactions in subjects treated for more than five years are significantly more likely to decrease (26.5 per cent) than to remain the same (9.5 per cent) or increase (8 per cent), as compared with subjects treated for one to five years in whom 24.5 per cent show a decrease while 22.5 per cent remain the same and 9.0 per cent show an increase. Evidently, therefore, the longer the treatment is continued, the more likely is the skin test reaction to decrease.

Upon clinical results: The data pertaining to the effect of duration of treatment upon clinical results, analyzed for varying periods of time,* are shown in Table V. It will be observed at once that the longer treatment is

TABLE V. EFFECT OF DURATION OF TREATMENT ON CLINICAL RESULTS OF TREATMENT (COMBINED GROUPS)

EFFECT ON CLINICAL RESULTS	1 YEAR*	2 YEARS AND OVER*	1 TO 5 YEARS	6 YEARS AND OVER	1 TO 10 YEARS	11 YEARS AND OVER
Good	6.5	66.5	39.5	33.5	65.5	7.5
Fair	1.5	19.0	12.5	8.0	18.5	2.0
Poor	2.0	4.5	4.0	2.5	6.0	0.5
Total	10.0	90.0	56.0	44.0	90.0	10.0

*p = <0.05 >0.02 (statistically significant).
(*Note:* All figures represent percentages.)

continued, the better are the results likely to be. Thus, patients treated for two years or more are significantly (vertical column marked with *) more likely to have good (66.5 per cent) or fair (19.0 per cent), rather than poor results (4.5 per cent) than is true of those getting treatment for one year only. By the time patients have been treated for five years or longer, the results of treatment generally are good and they become increasingly better because fewer patients have either fair or poor results. This suggests, therefore, that the longer treatment is continued, the better are the clinical results.

Upon dosage: The data pertaining to dosage, analyzed for varying periods of time, are shown in Table VI. Inspection of the latter shows that the longer

*The authors realize that hay fever patients who obtain satisfactory results are more likely to persist in treatment than those who do not, but this contingency has been considered in the statistical evaluation by comparing the results for single years (for example, one year, two years, etc.). For the sake of brevity, these detailed figures have been omitted.

the patient is treated, the more likely is the dosage to be 0.01 N or over. This is especially true when comparing subjects treated for one to five years, representing the majority (56 per cent), with those treated for more than five years (44.0 per cent). As shown in the table, patients treated for more than five years are significantly (vertical column, Table VI, marked with *) more likely (38.5 per cent) to have a dosage of 0.01 N or greater than less than 0.01 N (5.5 per cent) as compared with patients treated for one to five years (41 per cent compared with 15 per cent).

TABLE VI. EFFECT OF DURATION OF TREATMENT ON DOSAGE (COMBINED GROUPS)

EFFECT ON DOSAGE	1 YEAR	2 YEARS AND OVER	1 TO 5 YEARS*	6 YEARS AND OVER*	1 TO 10 YEARS	11 YEARS AND OVER
0.01 N and over	5.5	74.0	41.0	38.5	71.0	8.5
Less than 0.01 N	4.5	16.0	15.0	5.5	19.0	1.5
Total	10.0	90.0	56.0	44.0	90.0	10.0

*p = <0.02 >0.01 (statistically significant).
(Note: All figures represent percentages.)

From the analysis of the data relating to the duration of treatment, we may conclude that longer treatment brings higher dosage, better clinical results, and eventual decrease in the skin test reaction.

Effect of Method of Treatment (Preseasonal or Perennial).—While some of the data pertaining to the method of treatment employed already have been included or mentioned in our previous analyses, we felt that it might be of clinical interest to summarize these observations separately. In the first place, we found that the method of treatment apparently has no influence upon the change in skin test reaction, although a decrease in reaction is significantly more likely than an increase, regardless of the method of treatment. Likewise, little significant difference was noted in the results of treatment, regardless of whether the patient was treated preseasonally or perennially. However, the dosage distribution was different for the two groups. Thus, the proportion of cases receiving dosages larger than 0.01 N was significantly higher for the perennial than for the preseasonal group.

These results indicate, therefore, that there is no significant difference in the relationship of the method of treatment to the change in skin test reaction or to the clinical results obtained, but the pollen dosage is likely to be higher in the perennial group.

COMMENT

Pollen desensitization in seasonal hay fever long has been such a well-established and relatively unchanged procedure that allergists have only a rather general impression as to the effects of pollen therapy and its relationship to such factors as skin test reactions, dosage, and clinical results. However to the novice or beginner devoid of such experience, or to the questioning patient, definite information about these factors is lacking. Recourse to the

literature or to allergy textbooks provides conflicting opinion and only generalizations based on clinical experience. This uncertainty was especially evident in the answers furnished by allergists to our questionnaire survey. Therefore, when either the patient or the physician asks about the effect of pollen therapy, especially about the length of time it must be carried out and the expected clinical results, the answers often are indefinite because they depend mostly upon impressions gained from the clinical experience of the attending physician or allergist. We believe that the results of the investigations herein reported supply more definite answers to some of these questions and help to clarify existing uncertainty.

First, for example, let us consider the question of the effect of pollen desensitization upon the skin test reaction. The belief that pollen therapy causes a progressive decrease in the skin test reaction[1] was verified. However, the reaction seldom becomes negative; furthermore, such a decrease is more likely to take place in patients who have had long-continued treatment (more than five years), and in those patients who can take high doses of pollen extract. On the basis of these findings, one can conclude that a decrease in the skin test reaction is likely, but it would be unwise to use either this decrease as a criterion of subsequent dosage or the reduction to negative as a basis for the discontinuance of treatment.

Another observation resulting from our studies relates to the effect of pollen desensitization upon the final clinical results. From their accumulated clinical experience, allergists generally have agreed with the statements contained in publications by Walker[7] and Vander Veer and Clarke,[8] showing that pollen therapy generally is effective, especially if continued long enough. Our studies support these findings, since we have shown that the longer patients are treated, the better are the results, especially in patients who also showed decrease in their skin test reactions and in those who tolerated higher dosages of pollen extract (0.01 N or more). That this was true, regardless of whether treatment was perennial or preseasonal, is of interest because of the widely held belief, exemplified by statements in the allergy literature[9] and textbooks, that somewhat better results are to be expected after perennial treatment than after preseasonal treatment. In justification of the latter belief, however, it is our observation that a higher dosage level was reached by more patients in the perennial group than in the preseasonal group. This may be more apparent than real since, if the allergist finds that the patient cannot take higher dosages without an ensuing reaction, he usually will not recommend perennial treatment. The perennial group thus becomes a somewhat selective group of patients who can tolerate higher dosages; and, since they can do so usually for longer periods, the results in the perennial cases generally should be better. However, despite the general impression that results are better in the perennially treated cases, our studies show that the clinical results in both groups are about equal; therefore, so long as the

patient can take a high dosage and is treated for periods longer than five years, it makes little difference whether he is treated preseasonally or perennially.

Our observation that the duration of pollen desensitization favorably influences the clinical results is of interest in relation not only to pollen desensitization, but also to desensitization with other allergenic extracts (for example, molds, house dust, etc.) in conditions other than hay fever. Allergists sometimes find it difficult to decide how long they should continue desensitizing injections in patients who are feeling well and who are receiving their maximum tolerated dose, even at longer intervals. At times the patient settles it by discontinuing treatment, but in those willing to continue there is considerable uncertainty as to how long the injections should be continued or whether their continuance will bring about better results. There is uncertainty also as to how effective any type of desensitization is, whether in hay fever, asthma, or other allergic conditions. The results of our studies with pollen extracts lend further support to the clinical impression that long-term desensitization with allergenic extracts can be an effective therapeutic procedure.

Finally, our demonstration of the beneficial effect of high dosage also is of special clinical interest. While allergists generally try to administer the largest dose of pollen extract that the patient can tolerate, it sometimes is stated that the size of the dose does not necessarily influence the clinical result. That is, a patient could have a good clinical result even though his dosage is not high. While this may be true in some patients, our studies indicate that most patients do better with higher dosage.

SUMMARY AND CONCLUSIONS

A review of the case records of 200 ragweed hay fever patients who had been receiving treatment regularly, both preseasonally and perennially, and who had been retested at frequent intervals permits the following conclusions:

1. A decrease in the skin test reaction may follow pollen desensitization, but it is more likely in patients getting high pollen dosage and in those treated for more than five years, regardless of the method employed. It seldom becomes negative.

2. Pollen therapy in hay fever usually will produce good results, but this is more likely in patients whose skin test reactions decrease and also in patients who can take high doses of pollen extract.

3. The longer treatment is continued, the better the clinical results are likely to be.

4. The method of treatment, whether perennial or preseasonal, does not significantly influence the results obtained or the change in skin test reaction, although the dosage of pollen extract is likely to be higher in patients receiving perennial treatment because they are especially chosen for that group.

5. Our findings in these investigations of hay fever patients are helpful in relation to desensitization not only with pollen extract, but also with other allergenic extracts employed in allergic conditions other than hay fever.

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SENSITIVITY TO HAIR DYES

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THE use of ortho-, meta-, and para-diamino benzenes and aminophenols in combination with polyhydroxybenzenes in hair and fur dyes dates back to 1885.[1] Although an increasing number of persons use this type of hair dye, it is interesting to note that during the past twenty years the frequency of reports of sensitization to hair dyes has decreased. Many reasons can be advanced for the reduced frequency of sensitization. For one thing, the public has become educated to the existence of allergic reactions and consequently fewer persons with hair dye eruptions seek the aid of the physician. Furthermore, many women have learned of their hypersensitivity to particular dyes and after a first mild attack of dermatitis, or after a positive patch test, they avoid further exposures. The routine use of the patch test previous to the dying of the hair has become especially important. An additional factor is undoubtedly the greater purity of the chemicals used for hair dying. The importance of purification has been demonstrated by Sulzberger and associates,[2] who observed that purification of the dyes used in lipsticks reduced the number of allergic responses. Manufacturers have learned empirically to avoid the use of certain highly allergenic substances which produce natural hair color, and this also has reduced the number of allergic reactions. These facts suggest a revision of the problem and suggest that some of the older reports on this subject should be re-evaluated. Clinical reports which deal with pathologic changes that arise weeks or months after the last dye application should be discounted, if the symptoms reported do not conform with those observed under controlled conditions or in animal experiments with the compounds.

It may be emphasized that paraphenylenediamine and paratoluylenediamine do not penetrate the normal skin of man,[3] or mouse.[4] In fact, Sidi[5] found that only seven persons were sensitive to paratoluylenediamine among twenty persons sensitive to paraphenylenediamine. To obtain a similar number of sensitizations, as with 2 per cent paraphenylenediamine, a 5 per cent paratoluylenediamine was necessary.[6] Sulzberger and Rostenberg[7] tested 411 subjects suffering from dermatoses or having allergic skin diseases with a 2 per cent paraphenylenediamine in petrolatum in a closed patch test and obtained 6.3 per cent positive reactions. In 1930 the incidence of sensitivity to hair dyes was reported to be 1 in 20,000 to 30,000, while in 1952 an incidence of one case of dermatitis for about every 80,000 packages of hair dyes sold was recorded according to

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Received for publication May 18, 1956.

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Schwartz and Barban.[8] No sensitivity was observed by them in testing 1,000 persons with various shades of hair dyes. Schwartz[8, 10] attributed the drop in incidence to (1) increased use of the preliminary patch test, (2) purity of the components, and (3) greater care in preparing oxidizing solutions. However, Peck[9] disagreed with Schwartz's findings.

At the outset, we wish to emphasize that the experimental conditions of the tests we are here reporting, as well as the composition of the clinical material, were somewhat different from those of previous investigators.

COMPOSITION OF HAIR DYES AND THE MECHANISM OF THEIR ACTIONS

Hair dyes contain not only paraphenylenediamine. In some, paraphenylenediamine is replaced by paratoluylenediamine, which is considered less toxic. Commercial hair dyes are combinations of various compounds. These compounds in varying concentrations are dissolved in a base consisting of soap, detergent, fatty alcohols, glycols, glycerine, alcohol, free and bound ammonia, and perfume. The total content of these compounds in the dye varies from 20 to 50 per cent. The highest concentration of aromatic amines is present in the dark shades (especially in the black hair dye). In use, the dye solution is mixed with an equal volume of 6 per cent H_2O_2, or with solid urea peroxide. Pure aromatic diamines and polyhydroxy compounds are colorless and darken on oxidation. The alkaline peroxide mixture oxidizes the aromatic constituents of the dye and simultaneously bleaches the hair, thereby rendering it more receptive to the dyes. The dye components of low molecular weight penetrate the hair shaft, and are oxidized within the cortex to insoluble pigments. The pigments produced by oxidation with hydrogen peroxide are azines and oxazines bound to the protein of the hair.[11] Cross sections of the dyed hair show that the pigments are deposited in the cortex and not in the cuticle. The color produced by artificial deposition of pigment granules in hair and fur has the same natural appearance as that of the normal hair, which owes its color to melanin pigment deposition. Several authors believe quinone-diimine to be the first substance produced by the oxidation of para-hair dyes. This substance is highly irritating and sensitizing, has a characteristic odor, and is supposed to be the actual cause of the dermatitic reaction. Quinone-diimine is formed on oxidation of paradiamines in neutral solutions and in the absence of water. In the presence of water, this compound is rapidly decomposed. The presence of quinone-diimine has not been proved, nor has it been identified by its reactions or odor in the aqueous alkaline solutions of hair dyes after addition of peroxide.

Investigations by Cox[11] on the mechanism of oxidation of diamines have shown the presence of the Bandrowski base in the dye solution and on the keratin fiber. The actual coloring matter, the insoluble azine, was present inside the cortex combined with the protein and on the surface of the hair, but was not found in the dye solution. Contact with hair keratin, therefore, seems to be essential for azine formation. An aqueous solution or suspension of the Bandrowski base in the presence of peroxide does not penetrate the fibers and therefore cannot dye hair. For the dying process it is necessary that aromatic amines

penetrate the hair shaft and be oxidized therein. After dying fur with paraphenylenediamine and peroxide at an alkaline pH, the azine inside the dyed hair represented 27 per cent and the azine on the surface of the hair only 1 per cent of the diamine used. The Bandrowski base on the fur and in the solution or in suspension was equivalent to 45.1 per cent of the diamine. The unoxidized material, ammonia, and other reaction products accounted for the residual diamine. The azine and Bandrowski base formed on the surface of the fiber were easily removed by washing with soap and water. An excess of H_2O_2 did not improve dying, but caused the formation of more azine on the surface of the fiber. The azine formed contained, according to Cox, at least nine benzene rings. It was not extractable from the hair by pyridine or other solvents, and could be isolated only by dissolving the hair keratin in strong alkali. It did not melt below or at 300° C. An exazine of similar properties and structure was obtained after dying fur with para-aminophenol and peroxide at an alkaline pH.

PRESENT INVESTIGATIONS

The disparity between the assumed hazards of sensitization and the steadily increasing use of these dyes induced us to re-investigate the incidence of sensitivity. According to information received from one manufacturer of hair dyes used by hairdressers, one complaint was reported for approximately every 100,000 packages sold. From the description of symptoms, some claims appeared to be due to neither sensitization nor toxicity, but were nuisance claims. Clarification of this basic problem would benefit the dermatologist who usually is consulted only after allergies have developed.

MATERIAL AND METHODS

Patch tests were performed on 1,024 hospitalized patients who were free from any skin disorders and without history of allergic disease. As far as could be ascertained, none of the patients tested had previously used hair dyes. The persons tested were patients with tuberculosis, rheumatism, arthritis, and cardiovascular and neurological diseases—a very different group from that of Sulzberger and Rostenberg.[7] For the patch tests hair dyes used by hairdressers were utilized—again a material completely different from that used by Sulzberger and Rostenberg. To obtain the greatest possible response, the black dye was chosen since it has the highest concentration of aromatic amine. Every person was patch-tested with the following three solutions, each mixed with an equal part of 6 per cent H_2O_2:

Solution A: Black hair dye which contained paraphenylenediamine as the main ingredient.

Solution B: Black hair dye, which contained paratoluylenediamine as the main ingredient.

Solution C: Control consisting of the hair dye base and containing the same concentration of soaps, detergents, solvents, free ammonia, resorcinol, etc., but no aromatic amines.

Hair dyes A and B contained 2.2 per cent and 3.2 per cent of the paradiamines, and a total concentration of aromatic amines and related compounds of approximately 5 per cent. For the tests, only fresh bottles of dye, filled to the top and freshly mixed with hydrogen peroxide, were used within ten to fifteen minutes after mixing. The mixture was applied with a cotton applicator to the skin, which had been previously cleaned with benzene, and the test area was left uncovered to simulate the actual conditions of use (open patch test). The dye-peroxide mixtures were applied to the right and left antecubital folds, respectively, and the control solution was applied to the right retroauricular region. Patients were instructed not to wash the test area for at least twenty-four hours. The test areas were inspected after twenty-four and forty-eight hours and again after one week. Erythema alone was considered a 1 to 2 plus reaction, the formation of papules was scored 3 plus, and the formation of vesicles was considered a 4 plus reaction. The results are summarized in Table I.

TABLE I. SEX AND AGE DISTRIBUTION

AGE (YEARS)	MALE	FEMALE	A TOTAL	REMARKS
14 to 20	7	34	41	
21 to 30	35	77	112	One female positive to A
31 to 40	87	95	182	
41 to 50	98	83	181	
51 to 60	93	72	165	Two females positive to A One female positive to B One male positive to A
61 to 70	40	56	96	One male positive to B
71 and up	61	144	205	One female positive to A and B One female positive to B
No age given		46	46	
	421	607	1,028	

In a number of cases, including all those which gave a positive reaction to hair dyes, the tests were repeated two to four weeks later and in some instances were carried out a third time, two to three weeks subsequently (Table II). To determine the incidence of cross sensitization, four persons sensitive to hair dyes were tested with 2 per cent aqueous procaine solution (Table III).

TABLE II. REPEATS

AGE (YEARS)	REPEATED		REMARKS
	ONCE	TWICE	
15 to 20	12	6	
21 to 30	46	20	Includes 1 positive case
31 to 40	43	22	
41 to 50	22	11	
51 to 60	35	7	Includes 4 positive cases
61 to 70	42	1	Includes 1 positive case
71 and up	101	--	Includes 2 positive cases
Total	301	67	Includes 8 positive cases

RESULTS AND DISCUSSION

The studies performed on 1,024 persons affirm the practical value of patch testing. The data are summarized in Table I, which gives the incidence of persons allergic to hair dyes in relation to age and sex. Our findings indicate that

sensitivity to the two different hair dyes is highly specific. Three of the 1,028 persons (0.29 per cent) were sensitive to paratoluylenediamine type dye, four (0.39 per cent) to paraphenylenediamine type dye, and one (0.10 per cent) to both dyes. No persons reacted to the control solution. Three of the sensitive persons were male and five were female. All eight denied the previous use of hair dyes.

It should be made clear that the dye solution, the soap, and other components have a micellar structure, the single micelles consisting of 150 to 300 fatty acid or detergent molecules. The aromatic amines at alkaline pH are more soluble in the soap detergent micelle than in the aqueous solution; consequently, the physicochemical structure of the base acts as a deterrent to sensitization because the enmeshing properties of the micelles diminish the actual amount of diamines in contact with the skin even after drying. In addition to the previously mentioned explanations, the composition of the base of hair dyes may also be a factor in a lesser incidence of allergic responses to hair dyes. At present, the base contains 25 to 40 per cent soaps, detergents, solvents, humectants, etc. In former years 80 to 90 per cent of the dye base was water.

TABLE III. SENSITIVITY TO PROCAINE

CASE	AGE	PROCAINE
Female sensitive to A	28	Negative
Female sensitive to A	55	Positive
Male sensitive to B	65	Positive
Female sensitive to A and B	78	Negative

The number of persons sensitive to the black hair dyes seems also to be related to age. Only one of the 516 subjects less than 50 years of age (0.2 per cent) was positive to the black hair dye containing paraphenylenediamine. The remaining seven persons found sensitive were in the age group of 51 years or older. The conspicuous difference in sensitivity to hair dyes in persons over and under 50 years of age was unexpected since, as a rule, sensitization is supposed to be less common after the age of 50. According to Kern,[12] the skin shows diminishing reactivity to test materials with advancing age. Our results would indicate the contrary. Since the tested persons had disclaimed previous use of hair dyes, the positive reactions might be due to cross-sensitivity to related compounds. It is well known that persons allergic to local anesthetics of the procaine group or to sulfonamides or antihistamines or azo dyes, including Food, Drug, and Cosmetic certified dies,[13] may be sensitive to paraphenylenediamine. Our own tests with procaine (Table III) on four subjects sensitive to the black hair dyes seem to confirm these findings. Of four persons allergic to black hair dyes, two gave a positive reaction to procaine (cross-sensitization to para-amino-benzene group). The patch test, therefore, has important practical implications and is essential for every person who contemplates hair dyeing. It will help to eliminate persons cross-sensitized to aromatic amines or polyhydroxy aromatic compounds, by various drugs, azo dyes, or other compounds and thus further reduce the incidence of dermatitis due to hair dyes. Patch tests should also be made by the hairdressers before each dye application.

While we, ourselves, have not observed development of an allergic reaction upon repeated patch testing with para-dyes, this possibility, as well as cross-sensitization, should always be considered. The educational literature should stress the fact that even the slightest erythema may represent acquired sensitivity. Persons with injured or inflamed skin should not use hair dyes. Thorough shampooing of the hair after dyeing is of great importance. It removes from the surface of the hair and scalp all unoxidized dye or oxidation products which are capable of producing allergy. The dye pigment itself is inert and is lodged within the hair. Opinions regarding the persistence of permanency of sensitization are not uniform, although most dermatologists regard it as permanent.

The importance of the consistency of the reactions to patch test in relation to medicolegal problems and in workmen's compensation cases was pointed out by Witten and Shair.[14] In their carefully conducted experiment, they demonstrated that the standard patch test repeatedly performed is a highly reliable method for testing allergic contact type hypersensitivity and that consistent results are obtained on repetition. Our own interest was stimulated by an unpublished report by Sulzberger and Baer, who tested inmates of a penal institution with poison ivy extract. Several months later the same group was retested with the same extract and some of the previously strongly-reacting persons showed a decidedly diminished response. In our repeated patch tests (Table II), none of the persons found previously negative became sensitized on repetition. The repetitions of the patch test on the eight positive reactors gave the same response. In two, the reaction increased from 1 to 2 plus. In a third person, 28 years old, the patch tests were repeated twice, after one and five months, and showed gradually weaker reactions as compared to the first strongly positive test. A year later the same patient was retested with the same hair dyes three times at weekly intervals, and in all instances the patch test was negative. This is an exception to the rule that repeated exposures to a specific allergen in a sensitive person tend to increase rather than decrease the degree of reaction. However, graded dilutions of an allergen on repeated testing may show variations. Since standard concentrations have been used in our investigations, the consistency of our results is even more convincing in the light of Witten and Shair's work.

SUMMARY

Patch tests with oxidation (para-) hair dyes were made on 1,024 hospitalized male and female patients. Black hair dyes having the highest concentration of aromatic diamines were used. Two black hair dyes, one based on paraphenylenediamine and the other on paratoluylenediamine, were selected. Controls with the dye base which contained soaps, detergents, ammonia, resorcinol, and solvents were consistently negative. So far as could be ascertained, none of the persons, especially those found sensitive to the dyes, had used hair dyes previously. Of the eight persons found sensitive, three (0.29 per cent) reacted only to the black hair dye based on paraphenylenediamine, four (0.39 per cent) reacted only to the black hair dye containing paratoluylenediamine, and one

(0.10 per cent) reacted to both dyes. Age seemed to be related to incidence of sensitization. Only one person of the 516 patients 50 years old or younger was found to be sensitive to the paraphenylenediamine hair dye.

The remaining seven reactors were over 50 years of age and their positive skin tests with hair dyes might have been due to cross-sensitization to related compounds. In our experiments, two of four sensitive persons tested gave positive skin reactions with 2 per cent aqueous procaine solution. The patch test is essential, therefore, to any person contemplating hair dyeing.

The erythema in the positive cases was evident twenty-four hours after the application of the test solution, and in no instance was an increased or delayed reaction observed after forty-eight hours. The skin tests were repeated at intervals of from three to four weeks, once on 301 persons and twice on sixty-seven persons, including the eight sensitive persons. No change developed in the specificity of the reaction of persons sensitive to one or the other type of hair dye and no positive reaction appeared in previously negative cases. In the only patient under 50 years of age who was found to be sensitive to the dye, early repetition of the tests gave weaker reactions and repetition on three occasions a year later gave negative results.

The chemical composition of the presently used hair dyes, as well as the mechanism of the conversion of the para compounds into hair coloring pigments, is discussed.

We wish to thank Drs. Louis Leiter, Daniel Laszlo, E. G. Bloch, T. G. J. von Storch of Montefiore Hospital and Paul Kaufman and Jodh Sanghi of Goldwater Memorial Hospital for their cooperation in these studies.

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ARTHUS DESENSITIZATION

Studies of the Antibodies, Skin Reactivity, and Serum Proteins in Lasting Desensitization

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IT WAS reported previously that specific Arthus desensitization can be maintained for ten to fifteen days by large doses of antigen.[1] Culbertson,[2] Opie,[3] Fischel and Kabat[4] and co-workers have shown that the severity of an Arthus reaction in an actively immunized animal correlates closely with the amount of circulating precipitin. Preliminary analyses of the electrophoretic changes in those experiments[1] suggested the present study of the relationships between the serum proteins, skin reactions, and precipitin titers during repeated alternating periods of Arthus desensitization and recovery therefrom. Of particular interest was the production of lasting Arthus desensitization, coincident with immunologic exhaustion.

METHODS

Three rabbits were immunized by subcutaneous and intraperitoneal injections of egg white solution, until the precipitin titers were 10^{-6} or higher and the Arthus type skin reactions were 3+ or stronger. Skin reactions were elicited by intradermal injections of 0.1 c.c. of 1:20 egg white solution. Fresh areas were tested from time to time to rule out the possibility that apparent desensitization might be the *local* result of repeated injections of antigen into neighboring sites.

Skin reactions were read about twenty-four hours after intradermal injections of antigen. The 4+ reactions were deep red, firm, 3 to 4 mm. thick, with hemorrhagic necrotic central areas 10 to 15 mm. in diameter. These tended to heal with scabs. The indurated red areas were usually about 2 cm. or more in diameter. The 3+ reactions were similar, but with smaller and fainter hemorrhagic centers. They were less thick and firm and the diameters of the indurated red portion were usually 2 cm. or less. Scab formation was negligible. The 2+ reactions showed little, if any, central hemorrhage and the total diameters of the less red and less indurated areas were 1.5 cm. or less. The 1+ reactions were pink, slightly thickened, and approximately 1 cm. in diameter. Plus-minus reactions were faintly pink and barely palpable. Negative reactions had no redness or induration around the sites of the needle pricks.

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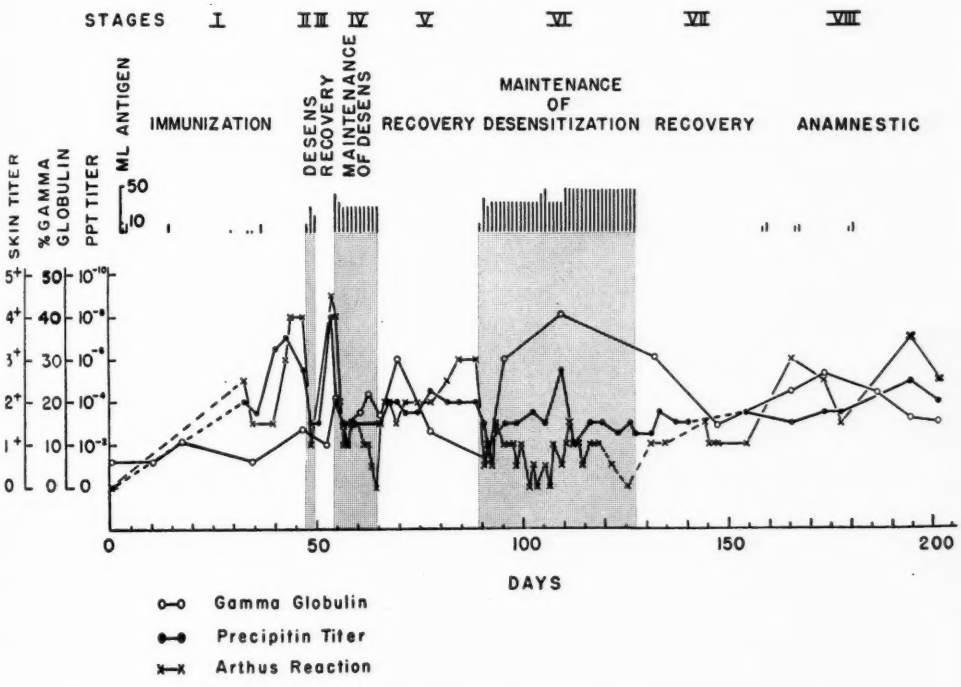
These studies were supported by Grant No. A-283 (C) M & I,5 from the National Institute of Arthritis and Metabolic Diseases, Bethesda, Maryland.

Received for publication Aug. 6, 1956.

Precipitin titers were obtained from decimal saline dilutions of egg white layered over antiserum, diluted 1:3 with saline, and read in fifteen minutes and in two hours. Desensitizing injections of 1:5 egg white were given in divided subcutaneous and intraperitoneal doses at intervals of one or two hours. Reasons for using egg white as the antigen and the ring test for precipitin titers have been discussed previously.[1] As controls, electrophoretic patterns and precipitin titers were done at regular intervals on two untreated rabbits. Skin tests could not be done on the control animals because antibodies and sensitization are readily produced by them.

The changes in the serum protein patterns were studied by determining the amount of total protein with a modified Linderström-Lang specific gradient[5] and the distribution of protein components by paper strip electrophoresis in a tent type Durrum apparatus.[6] The distribution of protein components of the serum was recorded with a densitometer. The quantitative proportions of the serum protein components were calculated from values obtained by photometric determination of the dye-protein complex eluted from the paper strips with 0.01 sodium hydroxide.

FIG. 1



The experiment was divided into eight stages (Fig. 1): Stage I, sensitization; Stages II, IV, and VI, desensitization; Stages III, V, and VII, recovery from desensitization; and Stage VIII, response to anamnestic injections. Desensitization in Stages II, IV, and VI was done by a preliminary subcutaneous

dose followed by daily intraperitoneal injections of 1:5 egg white until skin reactions were negative or equivocal and precipitin titers were 10^2 to 10^3. Desensitization was maintained arbitrarily for eleven days in Stage IV and for thirty-nine days in Stage VI.

During the recovery stages (III, V, and VII), no injections were given. These stages lasted until recovery of skin and precipitin titers reached predesensitization levels (Stage III), until lower recovery titers began to fall (Stage V), or until it seemed obvious that there would be no significant rise in the titers (Stage VII). The anamnestic stage (VIII), which lasted for forty-four days, consisted of three short series of antigen injections at intervals of seven and twelve days.

The serum-protein determinations were done twice a week. The skin reactions and precipitin titers were done daily during desensitization and the first few days of the recovery periods. They were done twice a week during the rest of the experiment, which lasted nearly seven months. Details of the antigen doses, duration of the stages, etc., are shown in Fig. 1.

RESULTS

Fig. 1 is a composite summary of the changes observed in the successive stages of the experiment, showing the averaged variations in gamma globulins, skin reactions, and precipitin titers in two rabbits. To simplify the curves, many of the estimations are not shown. The omitted values did not affect the shapes of the curves. Concomitant titers in the two rabbits differed very little. Slight differences in precipitin titers occurred a few times. There were four occasions when they differed by two decimal dilutions. Three concomitant gamma globulin levels in Stage VI showed appreciable differences which, however, did not alter the trend of the gamma globulin curve.

The changes in the third rabbit were similar to those described, until the animal died on the one hundred twentieth day. The values from this rabbit are omitted from Fig. 1 because it survived only slightly more than one-half of the experiment.

The following changes should be noted in the individual stages:

Stage I, sensitization: The gamma globulin, precipitin titers, and skin reactions increased.

Stage II, desensitization: The precipitin and skin titers and the gamma globulin levels fell promptly during this first desensitization.

Stage III, recovery: All three values recovered promptly and on the fourth day exceeded the predesensitization levels.

Stage IV, desensitization: The antigen injections reduced all three values and kept them low. The skin reactions were reduced more, proportionately, and the gamma globulins less than the precipitins.

Stage V, recovery: All values increased promptly during the second recovery period. Gamma globulin values were very high but fell

to predesensitization levels thirteen days after the last antigen injection. Neither the skin reactions nor the precipitin titers reached predesensitization (Stage I) or first recovery titers (Stage III).

Stage VI, desensitization: All values fell in response to daily desensitizing antigen injections. Progressively larger daily doses were used in the attempt to keep them low. Skin titers remained low but the precipitin titers and gamma globulin values did not.

The first sharp deviation from the rough parallelism between the three curves began four days after the daily desensitizing injections were resumed in Stage VI. The gamma globulins increased rapidly and markedly, and continued to rise for twenty days to a level of about 40 per cent of the total proteins (35 to 46 per cent). This was 2.5 times the predesensitization and nearly six times the presensitization levels. The gamma globulin values fell to about 28 per cent (25 to 34 per cent) while antigen injections were continued. The precipitin titers rose only slightly during the first fifteen days of this phase of marked increase of gamma globulins. In the next seven days there was a sharp peak in the precipitin titers in both of the rabbits, returning promptly to desensitization levels. The skin titers remained low throughout this period of desensitization.

Stage VII, recovery: Gamma globulins fell slowly to predesensitization levels during this thirty-day recovery period. Skin reactions and precipitin titers did not increase appreciably.

Stage VIII, anamnestic: Six anamnestic antigen injections during the final forty-four days of the experiment produced increases in gamma globulin, skin reactions, and precipitin titers.

Changes in Serum Proteins.—In the untreated rabbits the total serum protein ranged between 5.5 and 6.5 Gm. per cent. Four peaks could usually be identified in the electrophoretic pattern. The component with mobility similar to alpha$_1$ in human sera was generally absent. Thirty-nine estimations in each of the two control rabbits had variations in gamma globulins of less than 7 per cent. No precipitins were found.

In the treated rabbits the total proteins did not vary significantly during the experiment. During sensitization (Stage I) the alpha$_2$ globulins were markedly increased, although less so than the gamma globulins. During desensitization (Stages II, IV, and VI) the alpha and beta globulin bands split into several fractions. Fig. 2 shows individual electrophoretic patterns during the first six stages of the experiment.

DISCUSSION

The production of lasting Arthus desensitization and the comparison of changes in serum proteins with precipitins and skin reactions during prolonged drastic manipulations of the immune mechanism have not been reported previously.

An analysis of the sequence of events (Fig. 1) leading to lasting Arthus desensitization has been interesting, since several immunologic phenomena seem to be implicated. The so-called "negative phase"[7] of antibody depression is illustrated by the marked drop in precipitin and skin titers in the first desensitization (Stage II). A rebound to predesensitization titers has been observed before.[1-3] Recovery (Stage V) from the first period of maintained desensitization (Stage IV) was incomplete, presumably because larger desensitizing doses of antigen were used in the present experiment. This is in contrast to complete, but sometimes delayed, recovery in the previous report.[1]

There was no increase in skin reactivity during the thirty-day recovery period (Stage VII) following the prolonged second period of maintained desensitization (Stage VI). During this period, the enormous daily doses of antigen reached a total of 1,635 c.c. of 1:5 egg white solution, about 35 grams of protein. This was nearly thirty times the first desensitizing dose. The result seemed to be a state of "immunologic exhaustion," of which Arthus desensitization was one manifestation. This term is used to differentiate this condition, in which an active immune mechanism seemed to be overwhelmed, from immunologic paralysis[8] and immunologic unresponsiveness.[9, 10]

Immunologic paralysis[8] refers to the lifetime inability of mice to respond to specific pneumococcal polysaccharide injections, once their immune mechanisms have been "paralyzed" by *large* doses of that antigen. When compared with controls, about four times as much antigen is required to produce immunologic paralysis in mice which have been, but are no longer, immune to the antigen. Felton[8] attributed the "paralysis" to saturation of the reticuloendothelial system of mice with the polysaccharide antigen. Immunologic paralysis, then, differs from immunologic exhaustion in that the "paralysis" is produced before detectable antibody is formed.

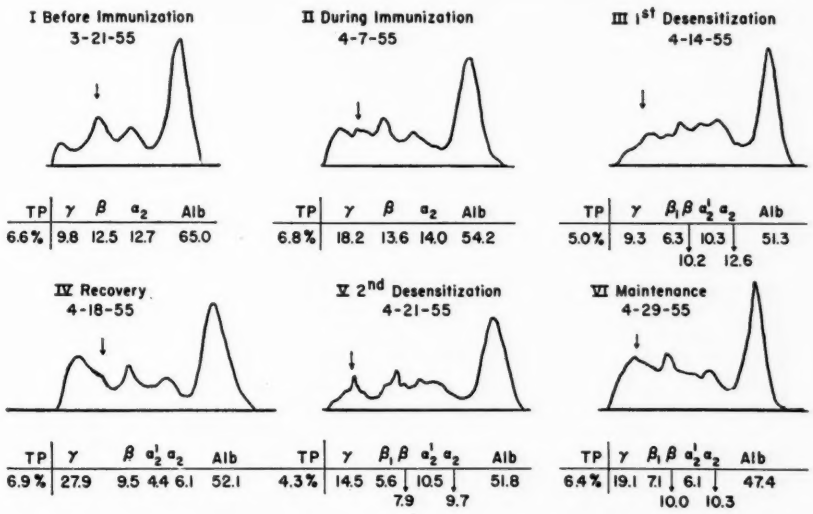
Immunologic unresponsiveness has been produced in newborn rabbits by *small* doses of antigen.[9] No antibodies were formed, supposedly because the young animals accepted the antigens, bovine and egg albumins, as isoproteins. Immunologic unresponsiveness has also been produced in adult and young rabbits[10] by *large* doses of the complex antigen, human plasma, and by the more refined antigen, bovine serum albumin. No antibodies to the major antigens were formed, but they were formed readily to the minor antigens present in human plasma and commercial bovine albumin. The phenomenon lasted indefinitely in young rabbits, but it lasted only about as long as the antigen could be detected in the adults, usually three to four months. Johnson, Watson, and Cromartie[13] felt that antibody formation was suppressed by saturation of antibody-forming sites with large doses of antigen. It is possible, by analogy, that the duration of the Arthus desensitization coincided with persistence of the antigen. It is unfortunate that the need for that information was not anticipated. The immunologic "exhaustion" observed in the present experiment differs from "unresponsiveness" by virtue of the abundant antibodies which were present before the overwhelming doses of antigen were given.

An analysis of the serum protein changes has been of interest also. The lack of change in the total serum proteins was not unexpected. Gamma globulin values did not correspond to the changes in the precipitin titers and skin reactions as closely as had been anticipated.

The striking increase in gamma globulins during the second period of maintained desensitization (Stage VI) is not readily explained. Some of this may have been due to circulating soluble antigen-antibody complexes. It is possible also, that a significant part of the gamma globulin did not contain antibody. Hanan and Ayama,[9] for example, showed that, after comparable antigen injections, the nonantibody gamma globulin values were as high (or higher) in immunologically unresponsive newborn rabbits as they were in control rabbits with normal antibody production. On the other hand, some of the increase in gamma globulin in this experiment may have been due to antibodies elicited by the minor antigens in egg white.[11] Dixon and Maurer[10] found antibodies to five human plasma fractions and to bovine gamma globulin in rabbits made immunologically unresponsive to human or to bovine serum albumins.

FIG. 2

ELECTROPHORETIC PATTERNS ON THE SERUM OF RABBIT DURING IMMUNIZATION AND DESENSITIZATION



The assumption seems reasonable that antibodies to the minor antigens of egg white were responsible, in part, for: (1) persistence of low precipitin titers during the desensitizing periods; (2) failure of gamma globulin, precipitins, and skin reactions to correspond more closely; (3) the transient peak in the precipitin titers in Stage VI; (4) splitting of the alpha and beta globulin bands in Fig. 2; and (5) anamnestic increases in gamma globulin and precipitin titers in Stage VIII.

The prolonged desensitization and apparent immunologic exhaustion which followed Stage VI did not prevent the subsequent return of immunologic activity following the anamnestic antigen injections in Stage VIII.

This experiment has emphasized the need for a pure antigen which would elicit only one antibody. Such an antigen would simplify attempts to appraise (1) the proportions of antibody to nonantibody globulin; (2) the time, dose, tissue, and specificity factors which determine when the antibody-forming mechanism will be depressed by a series of antigen injections; and (3) when or whether exhausted or unresponsive antibody-forming mechanisms will recover.

SUMMARY AND CONCLUSIONS

1. Periods of maintained Arthus desensitization were alternated with periods of recovery of sensitization in rabbits. Changes in serum proteins, precipitin titers, and skin reactions were observed during several such periods for 202 days.

2. Changes in total protein values were negligible. Alpha and beta globulin bands split into several small fractions during the desensitization periods.

3. Gamma globulin values were elevated after sensitizing anamnestic injections, and (after a transient depression) during a long period of desensitization. They were depressed by antigen injections, otherwise, and by omitting antigen injections for about two weeks.

4. There was a rough parallelism between the skin reactions and the precipitin titers. The gamma globulins paralleled the skin and precipitin titers at times, but diverged widely at others.

5. Desensitizing injections, which continued to depress skin reactivity and precipitin titers, did not continue to depress gamma globulin.

6. Prolonged Arthus desensitization was maintained by large daily doses of antigen, even such a complex antigen as egg white.

7. Repeated intermittent maintenance of desensitization led progressively to a stage of "immunologic exhaustion," characterized by failure of gamma globulin values, skin sensitivity, and precipitin titers to rise after antigen injections were stopped. This coincided with and is thought to be the first example of lasting Arthus desensitization.

8. The "immunologic exhaustion" was transient. Elevated precipitin titers, gamma globulin values, and skin sensitivity returned several weeks later in response to a series of anamnestic injections of antigen.

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PASSIVE CUTANEOUS ANAPHYLAXIS (PCA) IN THE GUINEA PIG

An Immunologic and Pathologic Study

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BIOZZI, Mene, and Ovary[1] studied the quantitative aspects of endothelial granulopexy induced by intradermal injection of histamine. Guinea pigs were given intrajugular injections of India ink and, after two hours, intradermal injections of histamine dilutions. Due to an increase in the permeability of the vessels, an effusion of plasma colored by India ink accumulated at the histamine-injected skin sites. The microscopic examination of the test sites revealed phagocytosis of the India ink particles by endothelial cells (granulopexy).

The authors included in their investigation an observation of granulopexy induced at the site of antibody-antigen reaction. A skin site passively sensitized with guinea pig antihorse-serum serum was challenged by intravenous injection of antigen (horse serum) and India ink. There was an efflusion of India ink at the antigen-antibody reaction site. The microscopic examination revealed minute hemorrhages, vascular lesions, and granulopeetic activity of cells in connective tissue, rather than in vascular endothelium only. Pretreatment with an antihistamine prevented both the effusion and the granulopexy. The authors concluded that granulopexy induced in guinea pig skin at the site of an antigen-antibody reaction is somewhat different from that induced by intradermal injection of histamine.

The antibody-antigen reaction in the skin of guinea pigs was termed passive cutaneous anaphylaxis (PCA) and tentatively attributed to the action of histamine liberated in the course of the reaction.[2]

Ovary and Briot[3] reported that an intracutaneous injection of the most minute amounts of rabbit immune serum (such as a 1:30,000 dilution of rabbit antiovalbumin serum) can still passively sensitize a site of guinea pig skin and induce a seepage of plasma. Determinations of precipitable antibody N (determined by the method of Heidelberger and Kendall[4]) indicated that about 0.003 meg. of precipitable antibody N was present in 0.1 ml. of 1:30,000 dilution of antiserum used for passive sensitization.

Various studies by Ovary,[5, 7] Ovary, Biozzi, and Mene,[6] Ovary and Bier,[8] and Biozzi, Halpern, and Benacerraf[9] established the basic observations on passive cutaneous anaphylaxis in the guinea pig as follows:

A skin site is passively sensitized with intracutaneously injected sensitive serum. The challenge with a mixture of antigen and dye is administered by an intravenous or intracardial route. An interaction of antibody and specific antigen is signified by a rapid accumulation of the dye in the subepidermal tissues surrounding the sensitized skin site.[7]

From The Institute of Allergy, Roosevelt Hospital, New York City.

Presented at the thirteenth annual meeting of the American Academy of Allergy, Los Angeles, California, Feb. 4-6, 1957.

Received for publication Oct. 5, 1956.

The induction of passive sensitivity after intracutaneous injecton of immune serum requires a latent period which was found to be inversely proportional to the amount of antibody used for passive transfer. The more potent the antiserum, the shorter the latent period before onset of passive sensitization.[9] This latent period cannot be reduced to less than thirty minutes between intradermal sensitization and subsequent challenge with antigen.[5] It has been noted that antihistaminics do not abolish the PCA reaction, but only modify it, preventing reactions at weakly sensitized sites and lessening the degree of reactions where more potent antisera induced the local sensitization.[6] The distinction between reversed Arthus phenomenon and PCA was postulated from experiments with antihistaminics which do not have any influence on reversed Arthus phenomenon but modify the PCA reaction. As another evidence aiding the differentiation of the two, it is stated that horse antityphoid polysaccharide immune serum is able to induce a severe reversed Arthus phenomenon in guinea pig, but is not effective in inducing local sensitization for passive cutaneous anaphylaxis.[8]

The passive sensitivity of guinea pig skin can be induced by the injection of certain human sensitive sera. Successful transfer of human diphtheria precipitating and nonprecipitating antisera and antityphoid and antibrucella convalescent sera has been reported by Ovary and Biozzi.[10] In our previous investigation,[11] using antisera of human origin, we have established that human sera of the *spontaneous* immediate (edema-erythema) type of sensitivity (against pollen antigens, horse epithelium, and horse serum) were not capable of sensitizing guinea pig skin. However, cutaneous reactivity was obtained with human sera of the *induced* immediate (edema-erythema) type of sensitivity against horse serum and with rabbit sera immunized with ragweed pollen extract. *Human blocking antibody*[14] against ragweed pollen failed to transfer and induce PCA in guinea pig.

The present study is an examination of the relationship of passive cutaneous anaphylaxis to immunological properties of various antibodies, and aims at the elucidation of the pathologic physiology of this cutaneous phenomenon.

EXPERIMENTAL TECHNIQUES

Passive Cutaneous Anaphylaxis (PCA).—Male albino guinea pigs, each weighing 350 to 400 grams, were used throughout the study. Abdominal skin, shaved with an electric clipper, was employed. Three to six intradermal sites were prepared, each with 0.1 ml. of sensitive serum or serum dilutions. Six hours later an intracardial injection of corresponding antigen-dye mixture was made. After thirty and sixty minutes, the reactions were read and considered positive if an accumulation of dye 7 mm. or greater in diameter appeared. Absence of dye seepage or discoloration of less than 7 mm. in diameter was considered negative.

For quantitative estimation of sensitizing activity, dilutions of sensitive sera were prepared with 0.85 per cent saline solution. The serum-saline dilution responsible for the minimal (7 mm. diameter) effusion of dye established the titration end point, that is, the maximal dilution of antibody capable of inducing reaction. The titration by dilution permitted fairly accurate estimation of sensitizing potency of investigated sera and facilitated quantitative comparisons.

In each test animal, a known positive reacting system in maximal dilution and normal serum were included as controls. Each serum was examined for PCA at least three times.

Schultz-Dale Phenomenon.—Isolated guinea pig ileum was used. Fresh segments of ileum were immersed in the sensitive serum for five hours at 37° C.; then they were washed and suspended in 0.85 per cent saline (37° C.). The reaction was considered positive if the challenge with specific antigen caused immediate contraction, followed by a refractoriness of the muscle to subsequent addition of the same antigen. Addition of histamine confirmed the remaining ability to respond, while a challenge with nonspecific antigen after a change of bath served as a control. Parallel experiments with ileum strips immersed in normal human or animal sera failed to produce reactivity upon addition of antigens.

Systemic Anaphylaxis.—Systemic sensitization of the guinea pigs was established by an intraperitoneal injection of 5 to 18 ml. of sensitive serum. The animals were challenged intracardially twice: with nonspecific antigen eighteen hours after sensitizing injection and six hours later with specific antigen. They were observed for signs and symptoms of anaphylaxis. The reaction was considered positive if dyspnea ended in death and the autopsy revealed emphysematous lungs.

Precipitation.—The technique described as agar gel diffusion Oudin,[12] as well as the classical test tube method of precipitation, was used.

Antigens.—

Passively sensitized animals: These were challenged intracardially with the proper antigens. The following amounts were used: 1 ml. of concentrated antigen extracts containing 100,000 to 200,000 protein nitrogen units* per milliliter; 0.2 ml. of horse serum; and 0.2 ml. of 5 per cent solution of the offending plasma protein in 0.85 per cent saline.

Passive cutaneous anaphylaxis: The challenging dose of antigen, as stated above, was mixed with 0.25 ml. per 100 grams of body weight of 1 per cent Evans blue solution just prior to injection.

Schultz-Dale experiments: Two-tenths milliliter of antigen extracts, horse serum, or plasma protein antigen solutions were used for each 10 ml. of saline constituting the suspension bath for tested intestinal segments.

Precipitation: Several dilutions of the antigens were employed.

Sensitizing Serums.—The various investigated sensitive sera, the sensitized species, and the offending antigens (respective test antigens) are listed in Table I. In the *spontaneous* human allergies of the immediate type (edema-erythema group) the antisera had skin-sensitizing property in at least 1:800 dilution in the Prausnitz-Küstner tests. The skin-sensitizing ability of human sera of the *induced* human immediate (edema-erythema) type of sensitivity was not demonstrable in dilutions exceeding 1:300 dilution.

Titrations of *sensitive sera of animal origin* indicated only a limited ability to sensitize human skin. For the purpose of the present investigation, only

*One protein unit contains 0.00001 mg. phosphotungstic precipitable nitrogen.

undiluted antiserum and 1:10 dilutions were employed to demonstrate human-skin-sensitizing property of animal antisera. The capacity to induce guinea pig skin sensitivity, reflected by PCA titers, did not relate proportionally to the human-skin-sensitizing activity.

Sera capable of transferring sensitivity to guinea pig skin induced positive PCA reactions in dilutions as high as 1:24,000 (rabbit antiovalbumin serum), 1:12,000 (human antihorse-serum serum), 1:6000 (guinea pig antibovine-albumin serum). Only very few ranged as low as 1:800 (human anti-Friedlander and antipneumococcus S.S.S. III-convalescent sera, rabbit anti-ragweed serum, and guinea pig antituberculopolysaccharide serum).

Serum Fractions.—These were derived from induced human antihorse-serum serum* and normal human serum and were employed for investigation of PCA phenomenon. Fractions designated as euglobulin, intermediate globulin, pseudoglobulin, and albumin were separated from serum on the basis of their solubility properties in ammonium sulfate solutions and then resolved by dialysis into water-soluble and water-insoluble constituents.[17] Electrophoretic pattern and mobility indicated that the fraction designated as *water-soluble euglobulin* is essentially gamma globulin.[13] This euglobulin dissolved in saline in about the same proportion as found in the original serum, demonstrated approximately 28 per cent of human-skin-sensitizing activity of the whole serum.[13] Its ability to sensitize guinea pig skin and induce a PCA reaction was reduced by about 50 per cent. The *water-soluble albumin* derived from the same horse-serum-sensitive serum failed to reveal activity in any of the above mentioned immunological tests.

Fractions designated as *normal human euglobulin* and *normal human albumin* were water-soluble constituents of fractions derived from normal non-allergic human serum (Krs.). They were isolated by ammonium sulfate fractionation and dialysis, as described above, for use as controls and as diluents as specified in the text.

EXPERIMENTAL FINDINGS

Table I shows the results of PCA in guinea pig and the contrasting immunological properties† of investigated sensitive serum as determined by testing their activities in terms of:

1. Passive transfer of sensitivity to human skin (skin-sensitizing property—term based on passive sensitizing activity upon human skin as demonstrable in Prausnitz-Küstner tests).
2. Passive transfer of sensitivity to guinea pig (muscle-sensitizing property—term based on passive sensitizing activity upon guinea pig smooth muscle as demonstrated by means of passive systemic anaphylaxis and Schultz-Dale phenomenon).

*Serum obtained from a patient who had suffered serum sickness following administration of tetanus antitoxin (horse serum).

†The nomenclature defining antibodies by their properties or functions is that suggested by Cook, R. A., and Spain, W. C.: Studies in Hypersensitiveness: A Comparative Study of Antibodies Occurring in Anaphylaxis, Serum Disease and Naturally Sensitive Man, J. Immunol. 17: 295, 1929.

TABLE I. COMPARATIVE STUDY OF IMMUNOLOGICAL PROPERTIES

SENSITIVE SPECIES	ANTIGEN	TYPE OF IMMEDIATE SENSITIVITY	DIRECT SKIN TEST, IMMEDIATE WHEAL, AND ERYTHEMA REACTION
Human	Ragweed pollen	Spotaneous	Pos.
	Timothy pollen		Pos.
	Horse epithelium		Pos.
	Horse serum		Pos.
	Pork		Pos.
	Peanuts		Pos.
	Egg white (hen)		Pos.
	Penicillin (e)	Induced	Pos.
	Tetanus antitoxin (test antigen: horse serum)		Pos.
	Pneumococcus T.III (test antigen: S.S.S. III)		Pos.
	Klebsiella friedländeri (f)		Neg.
	Eberthella typhi (g)		n.r. (a)
	Brucella melitensis (g)		n.r.
	Diphtheria toxoid (i)		Pos.
	Diphtheria toxoid (i)		Pos.
	Ragweed pollen extract (j)	Induced blocking	Neg.
Rabbit	Ragweed pollen extract	Induced	Arthus (k)
	Human albumin		Arthus
	Bovine albumin		Arthus
	Ovalbumin		Arthus
	Pneumococcus T.III (test antigen: S.S.S. III)		Arthus
Guinea pig	Bovine albumin	Induced (m)	Arthus
	Horse serum		Arthus
	Tubercle bacilli (n) (test antigen: TBB-polysaccharide) (o)		Pos. (p)

Key to symbols:

(a) *Abbreviations:* n.r. = not reported; n.d. = not done.
(b) Precipitation determined by agar gel diffusion (Oudin).[12]
(c) Published and discussed in our previous study.[11]
(d) Sensitivity to horse epithelium and horse serum.
(e) Four investigated specimens of this group were selected out of nineteen penicillin-sensitive sera because in these four instances only the patients from whom the bleedings were obtained suffered an anaphylactoid shock after administration of the drug, demonstrated positive immediate skin tests, and the sensitivity was passively transferrable to normal human skin. (Prausnitz-Küstner reaction in at least 1:200 dilution.)
(f) A filtrate of bacterial vaccine (derivative of *Klebsiella friedländeri* culture growth) was used as the test antigen.
(g) Published by Ovary and Biozzi.[10]
(h) Agglutination.
(i) Published by Kuhns and Pappenheimer.[16]
(j) Two nonallergic volunteers were given injections of low ragweed pollen extract; after repeated series of injections neither of the two demonstrated positive skin tests to the extract. The sera were obtained for use in testing of their blocking effect; 1 ml. of such immune serum "blocked" the activity of low ragweed pollen extract in the order of 1,600 protein nitrogen units.[17]
(k) Direct skin testing of the sensitized animals with antigen resulted in Arthus phenomenon characterized by hemorrhagic necrosis, verified by histologic examination.

3. Precipitation (precipitating property—term based on formation of specific precipitate upon interaction of antigen and antibody in vitro).
4. Agglutination (agglutinating property—term based on formation of specific agglutinate upon interaction of antigenic particles, that is, bacteria, and antibody in vitro).

OF VARIOUS ANTIBODIES IN SERA OF MAN AND ANIMALS

NO. OF SERA EXAMINED	IMMUNOLOGICAL PROPERTIES OF ANTIBODIES DETERMINED BY APPROPRIATE TESTS				
	PASSIVE TRANSFER IN HUMAN SKIN	PASSIVE SYSTEMIC ANAPHYLAXIS IN GUINEA PIG (SHOCK)	SCHULTZ-DALE PHENOMENON ON ISOLATED GUINEA PIG ILEUM	PRECIPITATION (b)	PASSIVE CUTANEOUS ANAPHYLAXIS IN GUINEA PIG SKIN
3	Pos.	Neg.	Neg.	Neg.	Neg. (e)
1	Pos.	Neg.	Neg.	Neg.	Neg. (e)
4 (d)	Pos.	Neg.	Neg.	Neg.	Neg. (e)
	Pos.	Neg.	Neg.	Neg.	Neg. (e)
1	Pos.	Neg.	Neg.	Neg.	Neg.
1	Pos.	Neg.	Neg.	Neg.	Neg.
2	Pos.	Neg.	Neg.	Neg.	Neg.
4	Pos.	Neg.	Neg.	Neg.	Neg.
4	Pos.	Pos.	Pos.	Pos.	Pos.
1	Pos.	Pos.	Pos.	Pos.	Pos.
1	Neg.	Pos.	Pos.	Pos.	Pos.
1	n.r.	n.r.	n.r.	Pos. (h)	Pos.
1	n.r.	n.r.	n.r.	Pos. (h)	Pos.
1	Pos.	Pos.	n.r.	*Neg.*	Pos. (g)
1	Pos.	Pos.	n.r.	Pos.	Pos. (g)
2	Neg.	Neg.	Neg.	Neg.	Neg. (e)
3	Pos.	Pos.	Pos.	Pos.	Pos. (e)
1	Neg. (l)	Pos.	Pos.	Pos.	Pos.
2	Pos.	Pos.	Pos.	Pos.	Pos.
2	Pos.	Pos.	Pos.	Pos.	Pos.
1	Pos.	Pos.	Pos.	Pos.	Pos.
2	Neg.	Pos.	Pos.	Pos.	Pos.
2	Neg.	Pos.	Pos.	Pos.	Pos.
pooled	n.d. (a)	Pos. (r)	Pos. (r)	Pos. (r)	Pos. (r)

(l) Rabbit antihuman albumin immune serum, when injected in human skin, did not produce a typical wheal and erythma reaction. After five to six hours the injected site became indurated. The infiltration was found maximal after twenty-four hours. This might be regarded as reversed Arthus phenomenon, although a confirming histological diagnosis was not made.

(m) Guinea pigs were immunized by series of subcutaneous injections of antigen with Freund's adjuvant.[18]

(n) Male guinea pigs were immunized by series of subcutaneous injections of autoclaved, washed, and lyophilized tubercle bacilli of human type (10 mg. of TBB in 1 ml. of paraffin oil biweekly for four to six weeks.

(o) Tuberculopolysaccharide S-4 for our investigation of the immediate type of reactivity of TBB-sensitized guinea pigs (direct skin tests and immunologic examinations of pooled serum) was kindly supplied to us by Dr. Dennis W. Watson, professor of bacteriology and immunology, University of Minnesota.

(p) Out of eighteen TBB-sensitized guinea pigs tested with TBB-polysaccharide S-4 six guinea pigs exhibited immediate erythma at least 15 mm. in diameter which became edematous within one to two hours. (Skin test doses of 10 mcg. of TBB-polysaccharide S-4 were used. Skin tests on normal guinea pigs failed to produce reactivity.) Twenty-four hours after skin testing with polysaccharide S-4 an induration was found at all sites irrespective of presence or absence of immediate response. Minute amounts of tuberculoprotein present in the polysaccharide may be regarded as responsible for this delayed type of reaction All eighteen TBB-immunized animals, when tested with 7 mcg. of P.P.D. (prepared from Parke, Davis & Company commercial tablets) demonstrated intensive delayed tuberculin reaction with central necrosis.

(r) P.P.D. used in identical control experiment failed to produce reactivity.

5. Blocking effect (blocking property—term based on behavior of these antibodies in skin tests[14] and must not be confused with the term "blocking" in serologic tests for Rh sensitization[15]).

Relation of Muscle-Sensitizing Property to PCA.—From the present experiments, it can be seen that the ability to induce PCA reaction is always parallel

to the ability of antibodies to sensitize guinea pig smooth muscle (compare passive systemic anaphylaxis and Schultz-Dale phenomenon with PCA reactions in Table I).

Relation of Skin-Sensitizing Property to PCA.—The human-skin-sensitizing sera of *spontaneous* immediate (edema-erythema) type and of the *induced* immediate (edema-erythema) type of sensitivity to penicillin, highly active in human skin by direct and indirect tests, were not capable of inducing guinea pig smooth muscle sensitivity; nor were they able to transfer to guinea pig skin (PCA).

Four hours' heating (at 56° C.) of human sera (induced antihorse-serum serum, antipneumococcus S.S.S. III-convalescent serum) as well as animal antisera, all capable of inducing human skin sensitivity, smooth muscle sensitivity, and PCA reaction, destroyed the human-skin-sensitizing property but did not alter the other two. Schultz-Dale experiments repeated with heated sera demonstrated the remaining muscle-sensitizing property, and repeated PCA titrations confirmed the transfer of sensitivity to guinea pig skin with no more than slight change of titration end points.

There is no indication that the human-skin-sensitizing property of antibodies, both spontaneous and induced, is responsible for or identical with that inducing guinea pig smooth muscle or guinea pig skin sensitivity (PCA).

Relation of Precipitins to PCA.—In our previous study,[11] using induced human antihorse-serum serum, we have reported that precipitins demonstrable by the Oudin method withstand heating at 56° C. for sixty hours. A marked decrease in precipitin content was evident after four hours' heating, as estimated by classical test tube method. Using a variety of precipitating sera, this observation may be confirmed. Most precipitins decrease markedly after heating for four hours; however, Schultz-Dale phenomenon remains positive and PCA reactions remain essentially unchanged as estimated by end point titration.

Human nonprecipitating antidiphtheria serum (included in Table I) has been reported to transmit smooth muscle sensitivity to guinea pig[16] as well as passive cutaneous reactivity.[10] This we have confirmed.*

Our experience with nonprecipitating guinea pig antibovine gamma globulin serum duplicates the latter report.

Eighteen guinea pigs were sensitized by a single intracardial injection of 1 ml. of 10 per cent bovine gamma globulin solution in saline. On the fifth, ninth, twelfth, fifteenth, and eighteenth days after injection of the offending antigen, 2 to 4 ml. of blood was withdrawn by cardiac puncture. Three guinea pig antibovine gamma globulin sera (one obtained on the fifth, two on the ninth day after sensitizing injection) were able to induce passive cutaneous reactions

*Subsequent to the first draft of this report, we have examined a human antidiphtheria serum supplied through the courtesy of Dr. A. M. Pappenheimer of New York University and another human antidiphtheria serum from our service at the Roosevelt Hospital. Both sera were nonprecipitating but gave a positive Prausnitz-Küstner reaction and a positive Schultz-Dale phenomenon, as well as positive PCA reactions, in dilutions as high as 1:256. The diphtheria toxoid concentrate (1950 Lf per milliliter) used in several dilutions as test antigen for precipitation, Schultz-Dale and PCA, was supplied through the courtesy of Dr. J. Freund by the Department of Health, City of New York. The purified diphtheria toxoid (50 Lf per milliliter) for skin testing as well as booster injections was donated by the Department of Public Health, Commonwealth of Massachusetts, Division of Biologic Laboratories.

(with end point titrations of 1:16, 1:64, and 1:128) and, when pooled, transferred smooth muscle sensitivity to normal guinea pig (positive passive systemic anaphylaxis) as well as to isolated intestinal segments (positive Schultz-Dale phenomenon). Precipitins in these sera were not detectable by classical test tube precipitation or by Oudin's agar gel diffusion.[12] Of these three animals, one died and the remaining two developed precipitins. Antisera of the other animals in the group either simultaneously transferred passive cutaneous reactivity to guinea pig skin and contained precipitins or simultaneously failed in both.

Furthermore, we were unable to elicit any anaphylactic phenomenon (passive systemic anaphylaxis, Schultz-Dale, or PCA), using rabbit anti-ragweed sera* to transfer sensitization and fraction 2 of low ragweed pollen extract[19] as the challenge. Precipitins in these sera, against fraction 2 of the low ragweed extract (determined by Oudin method), were repeatedly positive. Precipitation of normal rabbit serum by fraction 2, as controls, failed.

From the above-cited results, it seems that neither the passive cutaneous reactivity (PCA) nor passive muscle-sensitizing activity in guinea pigs is quantitatively or qualitatively related precipitin content of sensitive sera.

Fixation of PCA Reactivity.—Presence of normal serum (if used for preparation of sensitive serum dilutions) strongly interferes with fixation of antibody responsible for sensitization of guinea pig skin (PCA). Ovary and Bier,[20] using two rabbit antisera (antiovalbumin and antipneumococcus S.S.S. III), have demonstrated, by PCA end point titrations, that rabbit sensitive serum diluted in saline is capable of inducing passive cutaneous reactivity in about 50 to 100 times greater dilution than when diluted in normal rabbit serum.

Results of an extension and several variations of the quoted study are tabulated in Table II. Section A confirms the results reported by Ovary and Bier.[20] The variations of this model experiment (Sections B and C) ascertain that the intensity of passive sensitivity in guinea pig skin (PCA) following transfer of rabbit or human antisera *(heterologous serum transfers),* as well as guinea pig antisera *(homologous serum transfer),* is dependent upon quantitative relationship of antibody and serum proteins.

Experiments with sensitive euglobulin (essentially pure gamma globulin) isolated from induced human horse-serum-sensitive serum and fractions (euglobulin and albumin) of normal human serum (Krs.) are listed in Table II, Section D. In this section tabulated results reveal that the normal euglobulin, which is a fraction of normal human serum (Krs.), when in the same concentration as in the whole serum (1.17 per cent), inhibits the PCA reactions as much as the whole normal serum (Krs.). In contrast, the normal albumin, also a fraction of the same normal human serum (Krs.), in the same concentration as in the whole serum (4.28 per cent) does not inhibit the passive cutaneous sensitivity (PCA). In other experiments, to be reported later, the normal euglobulin solutions (Krs.) of double (2.34 per cent) and greater

*Rabbits were sensitized with alum-precipitated low ragweed pollen extract, containing fraction 1, fraction 2 and fraction 3.[19]

TABLE II. THE EFFECT OF NORMAL SERUM AND OF FRACTIONS OBTAINED FROM NORMAL SERUM UPON PASSIVE CUTANEOUS ANAPHYLAXIS

SECTIONS	ANTIBODY 0.1 ML. OF SENSITIVE SERUM INTRADERMALLY	ANTIGEN SIX HOURS AFTER PASSIVE TRANSFER OF ANTIBODY INTRACARDIALLY	DILUTIONS OF SENSITIVE SERUM PREPARED WITH	EVALUATIONS OF PASSIVE CUTANEOUS ANAPHYLAXIS IN GUINEA PIG SKIN (a) DILUTIONS 1: 4	8	16	32	64	128	256	512	800	1600	
A	Rabbit antiragweed	Ragweed pollen extract	0.85% saline	+	+	+	+	+	+	+	+	+	−	
			Normal rabbit serum	+	+	+	+	+	−	−	−	−	−	(b)
B	Human antihorse serum	Horse serum	0.85% saline	+	+	+	+	+	+	+	+	+	+	
			Normal human serum (Krs.)	+	+	+	+	+	+	−	−	−	−	(c)
C	Guinea pig antihorse serum	Horse serum	0.85% saline	+	+	+	+	+	+	+	+	−	−	
			Normal guinea pig serum	+	+	+	−	−	−	−	−	−	−	
D	Euglobulin derived from a human antihorse serum sensitive serum 0.5% solution in 0.85% saline (e)	Horse serum	0.85% saline	+	+	+	+	+	+	+	+	+	−	
			Normal human serum (Krs.)	+	+	+	+	+	−	−	−	−	−	
			Normal human euglobulin 1.17% (Krs.) (d)	+	+	+	+	+	−	−	−	−	−	
			Normal human albumin 4.27% (Krs.) (d)	+	+	+	+	+	+	+	+	+	−	

(a) Each PCA evaluation represents an average of three experiments.

(b) Similar results were obtained in experiments using rabbit antibovine-albumin immune system.

(c) Similar results were obtained in experiments using guinea pig antibovine-albumin immune system.

(d) The water-soluble normal human euglobin and normal human albumin isolated from normal human serum (Krs.) were dissolved in 0.85% saline in about the same proportion as found in the whole serum (euglobin 1.17%, albumin 4.27%).

(e) Serum was obtained from a patient who had suffered serum sickness following administration of horse tetanus antitoxin (induced type of sensitivity.

(4.68 per cent) amounts of the concentration used in the experiment tabulated in Section D (normal euglobulin, 1.17 per cent), showed that the inhibition of PCA increased proportionately to the increase of concentration of normal euglobulin (Krs.) solutions used for preparation of sensitive euglobulin dilutions.

From these experiments, it is apparent that the fixation of antibody gamma globulin to guinea pig skin is interfered with and the PCA reactions are inhibited when the content of normal serum gamma globulin is increased.

The passive sensitization of guinea pig skin (PCA) seems to be dependent upon competitive fixation of reactive antibody gamma globulin and serum gamma globulin. The ratio of antibody to serum globulin influences the degree of saturation of the skin receptors with reactive and nonreactive gamma globulins; the intensity of the PCA reaction varies accordingly.

Effect of Drugs on the PCA Reaction.—In an attempt to relate the mechanism of passive cutaneous anaphylaxis of the guinea pig to a mediatory action of substances possibly liberated in the course of antibody-antigen interaction, drugs known to influence allergic mechanisms were tested for their effectiveness upon the PCA reaction. Sensitive sites in guinea pigs were prepared with dilutions of 0.5 per cent induced human horse-serum-sensitive euglobulin, and six hours later the challenging doses of a mixture of horse serum, Evans blue, and either antihistamine, heparin,* hydrocortisone,† or epinephrine were injected intracardially.

The results are compiled in Table III and show that antihistamine, heparin, or hydrocortisone is able only to reduce the intensity of passive cutaneous anaphylaxis as evidenced by decrease of end point titers and reduction of size of positive reactions. The effectiveness of these three drugs seems to be limited by the role and/or the degree of importance of the partial mechanism in PCA antagonized by their pharmacodynamic activity. Administration of increased doses did not promote an increase of inhibition. The results recorded in experiments with epinephrine, a potent constrictor of the skin vessels, seem to emphasize the importance of vasoconstriction, a pharmacomechanic interference, with the development of PCA.

Gross Pathology and Histopathology of PCA Reaction.—Preliminary experiments and observations indicated a need for several minor modifications of the experimental procedure for macroscopic and microscopic observations of the development of passive cutaneous anaphylaxis in guinea pigs.

One per cent and 3 per cent saline dilutions of water-soluble euglobulin isolated from induced human horse-serum-sensitive serum were employed for transfer of sensitivity. Dilutions (1 per cent and 3 per cent) of normal human euglobulin derived from normal nonallergic serum served as nonreactive controls. The 1 per cent solution of sensitive gamma globulin, in amounts of

*Heparin, an inhibitor of Arthus[21] and Shwartzman phenomena,[22] prevents thrombus formation. Being an ingredient of hyaluronidase inhibitor,[22] it increases the viscosity of blood and diminishes the permeability of intracellular substances.[23]

†The water-soluble hydrocortisone has, as heparin, an antihyaluronidase activity and also a relaxing effect on guinea pig smooth muscle, antagonizing histamine.[24]

TABLE III. THE INHIBITORY EFFECT OF SOME DRUGS UPON PASSIVE CUTANEOUS ANAPHYLAXIS IN GUINEA PIGS

ANTIBODY 0.1 ML. OF SENSITIVE SERUM INTRADERMALLY	ANTIGEN SIX HOURS AFTER PASSIVE TRANSFER OF ANTIBODY	SIMULTANEOUSLY WITH ANTIGEN INTRACARDIALLY INJECTED:	EVALUATION OF PASSIVE CUTANEOUS ANAPHYLAXIS IN GUINEA PIG 60 MINUTES AFTER CHALLENGE (a) DILUTIONS 1:						
			0	10	100	200	400	800	1000
Euglobulin derived from a human horse-serum-sensitive serum 0.5% solution in 0.85% saline (f)	0.2 ml. horse serum	Control without drug	+	+	+	+	+	+	−
		3 mg. antihistamine (b)	+	+	+	+	−	−	−
		5 mg.	+	+	+	−	−	−	−
		7 mg.	+	+	+	−	−	−	−
		11 mg.	+	+	+	−	−	−	−
		3 mg. heparin (c)	+	+	+	+	+	−	−
		5 mg.	+	+	+	+	−	−	−
		7 mg.	+	+	+	+	−	−	−
		9 mg.	+	+	+	+	−	−	−
		10 mg. hydrocortisone (d)	+	+	+	+	−	−	−
		15 mg.	+	+	+	−	−	−	−
		20 mg.	+	+	+	−	−	−	−
		25 mg.	+	+	+	−	−	−	−
		0.01 mg. adrenalin (e)	+	+	−	−	−	−	−
		0.1 mg.	−	−	−	−	−	−	−

(a) Each PCA evaluation represents an average of three to five experiments.
(b) Chlor-Trimeton (chlorprophenpyridamine) maleate, Schering Corporation.
(c) Isotonic solution of Liquaemin sodium (brand of heparin), Organon, Inc.
(d) Intravenous Solu-Cortef (hydrocortisone sodium succinate). The Upjohn Company.
(e) Epinephrinehydrochloride (also containing chlorbutanol and sodium bisulfite), Armour Laboratories.
(f) Serum was obtained from a patient who had suffered serum sickness following administration of horse tetanus antitoxin (induced type of sensitivity).

0.05 ml. per site, was able to induce minimal PCA (7 mm. in diameter) in 1:800 dilution; the 3 per cent solution, 0.05 ml. per site, titrated as far as 1:2,400.

Two skin sites were prepared with 0.05 ml. on each side of the abdominal wall, one with the sensitive gamma globulin and the other with the corresponding dilution of normal gamma globulin. The initial sensitizing intradermal injection bleb was 2 to 3 mm. in diameter. It rapidly flattened and spread out. In one hour the margins became diffuse. By the second hour it was difficult to identify the site of the injection. After three or four hours the skin had a normal appearance. Whenever, due to individual intolerance of the guinea pig skin, an irritant effect of the injected material occurred, such as slight swelling or mild erythema, the animal was eliminated from further experimentation.

The histology of the passively sensitized site before challenge with antigen was that of a very mild, nonspecific, inflammatory reaction with its maximum at the third hour after the injection. It consisted mainly of polymorphonuclear leukocytes, both diffuse and perivascular. This very mild infiltration was confined solely to the initially injected site and the adjacent area, totaling about 5 to 6 mm. in diameter. Toward the sixth hour, the infiltration had undergone major involvement, and it was of minimal density eight hours after the initial injection. On the basis of the microscopic findings, it was decided to begin the further observation eight hours after the initial intradermal injection of sensitizing material on both *the quiescent (before challenge) sensitized sites and the reacting (after antigen-dye challenge) sensitized sites.*

The skin on the belly, including the subcutaneous connective tissue and subdermal layer containing cutaneous blood-supply vessels, was cut along the entire midline, carefully separated from the abdominal musculature, and reflected for about 3 to 5 cm. The parallel venules and arterioles, as well as capillaries, of the *quiescent sensitized site* (that is, before challenge), viewed by means of thirtyfold surface magnification, were of unchanged size, the venules being slightly larger than the corresponding arterioles. There was no obstruction of the circulation due to contraction of the vessels or obliteration of the lumina by leukocytes or erythrocytes. At this point, the animal was sacrificed and speedily drained of blood. The subdermal layer was stripped away and the skin and subcutaneous connective tissue were exposed. The blood-free vessels in a quiescent sensitized site collapsed and became grossly invisible in the connective tissue. Only a faint yellowish discoloration, about 3 to 4 mm. in diameter, indicated the initial intradermal injection bleb.

The same technique was employed for observation of changes in the *reacting sensitized areas* (that is, after challenge with antigen-dye mixture). For the photographs of reacting areas on the reflected skin, the animals were sacrificed and bled out at times specified in the legends for the respective illustrations, and the subdermal layer was removed from the skin and subcutaneous connective tissue as described above.

The major changes in the *reacting sensitized area* occur within ten minutes after the intracardial challenge with antigen-dye mixture. Fifty to ninety seconds after the challenge, an elliptical area of the skin (about 40 by 20 mm.), surrounding the site of initial intradermal injection of sensitized material, flares up. The skin becomes vividly erythematous for several seconds (Plate I, *A*). The hyperemic flare is caused by an instant distention of capillaries and venules (Plate I, *B*). Arterioles seem not to take part in the reaction, and neither dilate nor contract. The flow of blood into the reacting area continues, and the dilation of venules and capillaries increases as more

Fig. 1.

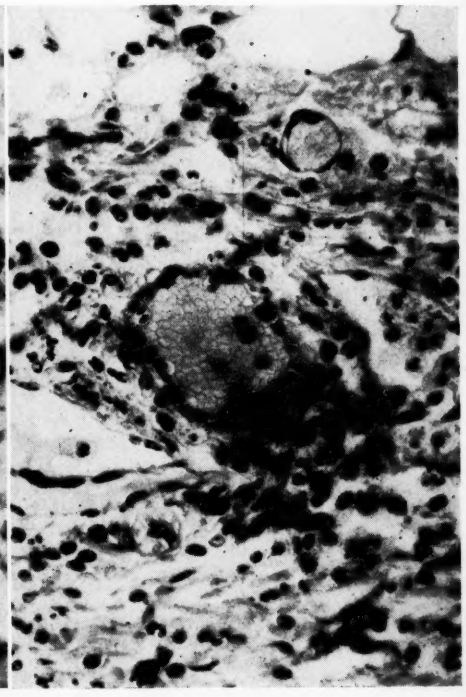

Fig. 2.

Fig. 1.—A venule, congested with erythrocytes and a patent, not contracted arteriole. The perivascular infiltration by degenerate leukocytes is a residue of a moderate, nonspecific inflammatory reaction caused by the intradermal injection of sensitizing gamma globulin eight hours before the challenge and the onset of the PCA reaction.

Fig. 2.—A capillary and a venule, both maximally congested with erythrocytes, shortly after the onset of the PCA. The perivascular infiltration rapidly increases by diapedesis of leukocytes.

blood cells enter (Figs. 1 and 2). The movement of erythrocytes in the engorged veins and capillaries is almost completely halted at the point of highest congestion. White blood cells accumulate at the walls of the venules, at times in layers several cells thick. Some leave the lumen and enter the surrounding tissues by rapid diapedesis, some adhere to the endothelial lining of the venules, and some form clusters among the congested erythrocytes (Figs. 3 and 4). The appearance of leukocytic clusters coincides with a short but

Plate I.—Illustration of chronological development of PCA reactions in guinea pigs. Gamma globulin, derived from human induced horse-serum-sensitive serum, was used for passive transfer of PCA sensitivity; 0.05 ml. of 3 per cent dilution was injected intradermally into guinea pigs *A* and *B* and 0.05 ml. of 1 per cent dilution was injected in guinea pigs *C* through *H*. (Nonreactive gamma globulin derived from human nonallergic serum served as control. See guinea pigs *A* and *G*.) Eight hours later the antigen (horse serum) with Evans blue was administered intracardially and the development of PCA reactions photographed at intervals as follows:

A, PCA on the skin surface 50 to 90 seconds after challenge.
B, PCA on the reflected skin 50 to 90 seconds after challenge.
C, PCA on the skin surface 70 to 120 seconds after challenge.
D, PCA on the reflected skin 70 to 120 seconds after challenge.
E, PCA on the skin surface 3 to 5 minutes after challenge.
F, PCA on the reflected skin 3 to 5 minutes after challenge.
G, PCA on the skin surface 10 to 12 minutes after challenge.
H, PCA on the reflected skin 10 to 12 minutes after challenge.

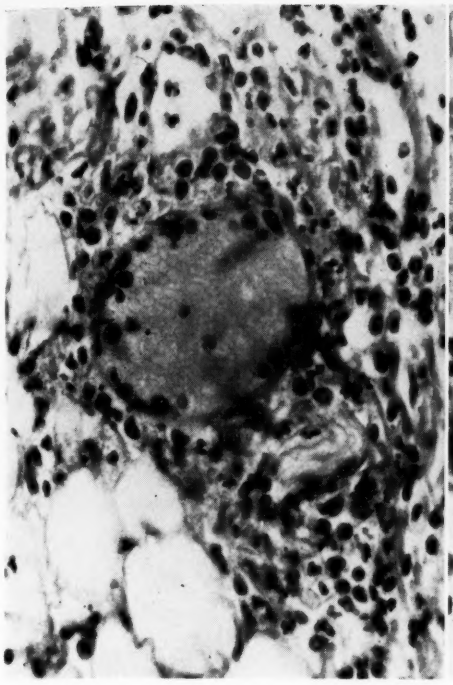

Fig. 3.

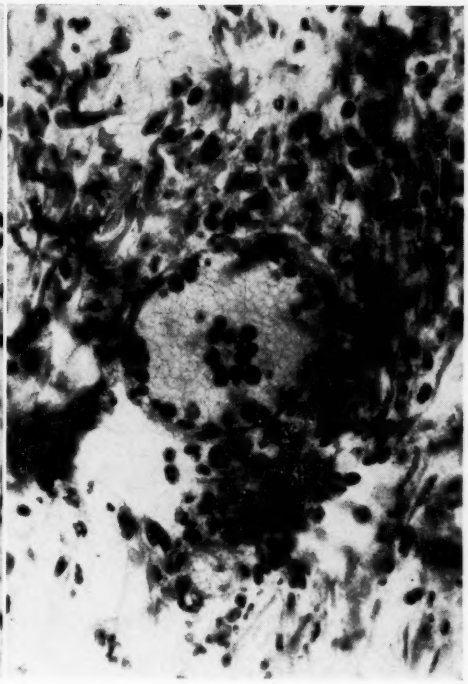

Fig. 4.

Fig. 3.—A maximally dilated venule, congested with erythrocytes. Leukocytes adhere to the endothelial lining. Note the interstitial edema with infiltration.

Fig. 4.—A venule with changes as in Fig. 3. In addition to a number of white blood cells adherent to the wall, there is a cluster of leukocytes in the center of the congestion.

Fig. 5.

Fig. 6.

Fig. 5.—A venule with an aggregate of erythrocytes. The whole endothelial lining is lifted away from the musculature of the venule wall by intruded leukocytes.

Fig. 6.—No such changes are found in the concomitant arteriole. The lumen is patent to blood flow. There is no adherence or clustering of leukocytes. The endothelial lining is free of changes.

complete arrest of flow movement in the affected venules. This aggregation and compression of erythrocytes reaches its maximum four to six minutes after the challenge.

The endothelial cells of the venules are gravely damaged. The cellular protoplasm is swollen and the nuclei are pyknotic and achromic. In some venules, the leukocytes creep under the damaged endothelium and separate the endothelial lining from the musculature of the vessel wall (Fig. 5). No such changes can be observed in the concomitant arterioles (Fig. 6).

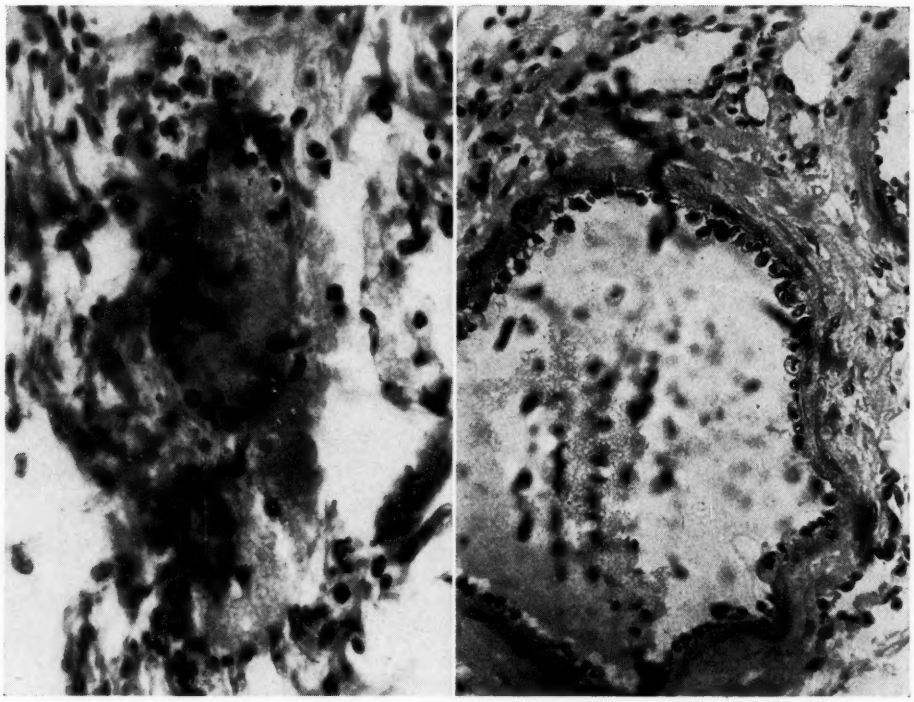

Fig. 7. Fig. 8.

Fig. 7.—An engorged venule surrounded by a mass of extravasated erythrocytes caught in adjacent connective tissue.

Fig. 8.—A venule freed of erythrocytic congestion. The endothelial lining is covered by adherent white cells.

At the time of the initial flare-up (fifty to ninety seconds after challenge) and throughout the above-described process to maximal congestion, extravasations of erythrocytes occur alongside the venules. The intensity and number of these extravasations are proportionally related to the sensitizing dose of the material used for passive sensitization of the skin site. The extravasations are markedly more numerous in the reacting areas sensitized with 3 per cent solution of sensitive gamma globulin than in those sensitized with 1 per cent solution (Plate I, compare *B* and *D*). The circulation in the venules interrupted by extravasation of erythrocytes ceases and the blood cells are caught permanently in the connective tissue surrounding the venule (Fig. 7).

Simultaneously with (or a few seconds after) the initial hyperemic flare, an exudation of the blue-colored plasma begins on the periphery of the reaction, demarcating the outer areas of sensitization (Plate I, *C* and *D*). Five minutes later, the blue effusion has spread centripetally and forms a band around the reacting site (Plate I, *E* and *F*).

A difference in intensity and speed of changes, within the outer and central portions of the reacting area, affecting the flow rate of blood, may be responsible for the early peripheral and delayed central exudation. It would seem that the flow of blood in the marginal areas is slowed down by dilatation of the venules and capillaries, but not brought to a complete stop by clustering and adherence of leukocytes and peeling of the endothelial lining, as noted in the venules of the central portion of the reacting area (the outer exudation starts immediately after the onset of the reaction). The flow of blood in the central areas is almost completely halted and, for a short period of time, is arrested; thus, the central exudation is delayed during the time of circulation stoppage.

Fig. 9.—Six hours after the initial flare, the subcutaneous edema is of maximal thickness (about 3 to 4 mm.). The cellular infiltration is now homogeneously distributed throughout the edematous connective tissue. There are a venule and concomitant arteriole, both free to flow of blood.

Approximately four to six minutes after the beginning of the reaction, the masses of red blood cells in the venules of the central area break apart and begin to move. The erythrocytic aggregates, as well as the clusters of white blood cells occluding the lumina and thickly covering the endothelium, disappear in the circulation. With the blood flowing through the dilated capillaries and veins, many of them stripped of their endothelial lining, voluminous exudation begins instantly. Within several minutes, the entire reacting area is covered and the tissues are soaked with blue-colored plasma (Plate I, *G* and *H*).

The preparation of the skin for the photographs is described above. The dilated veins, freed of obstructing masses of erythrocytes and leukocytes, are

emptied of circulating blood and are collapsed in the subcutaneous connective tissue (Fig. 8). Veins permanently blocked by extravasation of red cells and those, at the time of exposition after the animal has been sacrificed, still plugged and engorged by erythrocytic and leukocytic thrombi, show in the Kodachromes of PCA reactions (Plate I, *B, D, F,* and *H*).

For six hours after the initial flare-up, the subcutaneous interstitial edema gradually increases in thickness to 3 to 4 mm. (Fig. 9) and centrifugally in size to 65 by 35 mm. Thereafter, no apparent increase of edema occurs. During the time of actual reaction (first ten minutes) and thereafter, a great number of polymorphonuclear and mononuclear white blood cells leave the circulation by rapid diapedesis. The thick, essentially perivascular infiltration (Fig. 10) spreads homogeneously throughout the reacting site simultaneously with increase of the interstitial edema fluid, entering into spaces between the dissociated connective tissue fibrils (Fig. 9). In the six- to eight-hour lesions, many leukocytes are found to be already degenerate. No further major quantitative increase or qualitative change (such as eosinophilic or

Fig. 10.—A venule with a mass of leukocytes adhering to the endothelium, obstructed by an erythrocytic aggregate, and a concomitant arteriole free of such changes. The vessels are surrounded by a dense perivascular infiltration. The infiltrative cells begin to spread into spaces between the dissociated connective tissue fibrils.

mononuclear reactions) of the infiltration becomes evident. In twelve to thirty-six hours the edema undergoes a progressive and total resorption without any tissue damage, such as necrosis of connective tissue or epithelium of the skin. In thirty-six-hour lesions there are still some pyknotic polymorphonuclear leukocytes. There is also a fresh, diffuse, but sparse, infiltration by mesenchymal cells, histiocytes, and local proliferative-reparatory reaction around veins obliterated by extravasation of erythrocytes. However, within three days after the reaction, all remnants of edema and cellular elements completely disappear, leaving no trace of damage.

Comment on the Pathology of PCA Reaction.—Passive cutaneous anaphylaxis in the guinea pig is a vascular phenomenon with dominant venous and capillary manifestations. It is the result of an interaction *(occurring simultaneously at all sensitive points)* between passively fixed antibody and corresponding antigen brought in via circulation. The fixation of antibody occurs during the resorption of sensitizing material in the cutaneous network of

capillaries and venules, and is conditioned by binding affinity of globulins to guinea pig skin. (There seem to be globulins which are not bound, such as human blocking antibody,[11] and horse,[8] bovine, and goat gamma globulins.[25]) During the PCA reaction, a hyperemic flare develops as a result of dilatation of venules and capillaries. Arterioles do not seem to contract or dilate. Possibly the hydrodynamic pressure in the venules and capillaries increases due to dilatation of the lumina,* and as a result the damaged vessel walls become maximally pervious to plasma.

In contrast to our findings stands the work of Abell and Schenck,[27] who observed microscopically a reaction in the ear of an actively sensitive rabbit after introduction of antigen (Arthus phenomenon). Arteriolar contraction with obliteration of the lumina and shutting-off of the circulation in the reacting area was noted. Their observation is extended by Benacerraf and Kabat,[28] who noted in passive Arthus phenomenon in guinea pig skin a contraction and fragmentation of arterioles after intradermal injection of antigen. In the PCA there is no evidence of a protracted spastic constriction of arterioles and, also contrary to the observations by Abell and Schenck,[27] there is a maximal dilatation of venules and capillaries. The intravenous changes in the PCA (adherence and clustering of leukocytes, endothelial damage, congestion, and extravasation of erythrocytes) are similar to: (1) those seen by Lecomte and Hugues[29] in mesenteric vessels of sensitive rabbits and guinea pigs after contact with antigen, (2) those reported by Irwin, Weille, and Burrage[30] during systemic anaphylaxis in the guinea pig, and (3) those reported by Abell and Schenck[27] in the ear venules of sensitive rabbits after antigen has been repeatedly instilled.

To dispel doubts and ascertain a distinction between PCA (an interaction of intradermally *fixed* antibody and circulating antigen) and reversed Arthus phenomenon (an interaction of circulating antigen and intradermally *instilled* antibody), both reactions were demonstrated with identical antisera in the same guinea pig.

On one side of the belly a skin site was injected intradermally with antiserum (0.1 ml. of 0.5 per cent or 1.5 dilutions of induced horse-serum-sensitive gamma globulin). Eight hours later, antigen (0.5 ml. of horse serum) and dye were injected in the circulation. PCA reaction developed. Next, the identical antiserum was injected intradermally on the other side of the belly. At this skin site, a reversed Arthus phenomenon (severe with 1.5 per cent, moderate with 0.5 dilution) developed within six to eight hours. The changes at the reversed Arthus phenomenon site were similar to the changes described for Arthus phenomenon† resulting in a necrosis of connective tissue and epithelium within eight to thirty-six hours[31, 32] contrary to the evanescent changes at the site of PCA reaction.

*Bernoulli's theorem[26] states that along a stream line where the velocity of a fluid is high (narrow lumen) the hydrodynamic pressure on the wall is low or negative, where the velocity is low (wide lumen) the pressure is high.

†That is, blanching of the reactive site for three to four hours' contraction and fragmentation of the arterioles[27, 28] centrifugally developing edematous infiltration,[31] quantitative and qualitative changes of cellular infiltration.[31, 32]

SUMMARY

1. In view of present experiments, the immunologic property of antisera to induce passive cutaneous sensitization to guinea pig skin is attributed to the guinea pig smooth-muscle-sensitizing antibody, not conditioned by presence of precipitins. The human-skin-sensitizing antisera property (of human or animal origin) is neither related to the muscle-sensitizing property nor responsible for the induction of passive sensitization of guinea pig skin (PCA).

2. The fixation of antibody responsible for passive cutaneous reactivity is dependent upon competitive fixation of reactive antibody gamma globulin and serum gamma globulin. The ratio of antibody to serum globulin seems to determine the degree of saturation of the skin receptors with reactive and nonreactive gamma globulin.

3. The efficacy of antihistamine, heparin, and hydrocortisone to inhibit passive cutaneous anaphylaxis in guinea pig is limited. Administration of increased doses does not promote increased inhibition. Nontoxic doses of adrenaline are the most effective. The vasoconstricting effect of adrenaline appears to emphasize the vasodilation as an important partial mechanism in passive cutaneous anaphylaxis.

4. Passive cutaneous anaphylaxis in the guinea pig is a manifestation of an interaction between a passively fixed antibody and circulating antigen simultaneously at all points of sensitivity. It is a vascular phenomenon with dominant venous and capillary manifestations, rather than an arteriolar response as in Arthus phenomena. This is evidenced by the macroscopic observations and the microscopic findings in passive cutaneous anaphylaxis.

The authors wish to thank Dr. Walter Brandes, for assistance in the preparation of the histologic sections, and Dr. Paul Klempner, for advice concerning the pathologic discussion.

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STUDIES IN ALLERGENICITY OF EXTRACTS PREPARED FROM SPORULATING AND NONSPORULATING ALTERNARIA TENUIS

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THE studies presented herewith were carried out as part of a cooperative investigation of the allergenic activity of extracts prepared from cultures of both sporulating and nonsporulating *Alternaria tenuis.*

The extracts of alternaria employed were prepared in the laboratories of Dr. Marie B. Morrow of the University of Texas in collaboration with Dr. Homer E. Prince, Houston, Texas, and in those of the Division of Immunology of the Washington University School of Medicine.

The activity and cross-reactivity of both sets of extracts were compared by measuring the degree of neutralization of skin-sensitizing antibody.

DESCRIPTION OF EXTRACTS

A. *Prince-Morrow (PM) Extracts.*—The extracts supplied by the University of Texas laboratory were labelled "*Alternaria tenuis,* sporulating, #33-259-2/3/55" and "*Alternaria tenuis,* nonsporulating, #33-255-2/3/55." Both extracts, in the concentration of 1-50, contained 50 per cent glycerin and were clear and of dark brown color. These extracts had been prepared by the methods of culture, extraction, fractionation, and re-extraction devised and described by Prince and his associates;[11] their techniques differed significantly from those used in the Washington University laboratory.

B. *Washington University (WU) Extracts.*—The extract of sporulating *Alternaria tenuis* was derived from a culture obtained from the American Type Culture Collection (#11612). The extract of nonsporulating *Alternaria tenuis* was prepared from a subculture of the original from which the University of Texas nonsporulating extract (PM-NS) was derived. No spores were found upon microscopic examination of the mats grown from cultures of this nonsporulating form during the preparation of the Washington University nonsporulating extract (WU-NS).

Culture, desiccation, and defatting: All cultures were transferred from their original stock slants to fresh agar slants prepared from Bieberdorf solution, a synthetic, nonprotein medium,[1] to which agar was added to a final concentration of 1.5 per cent. After growth of one week, Fernbach flasks containing approximately 800 ml. of Bieberdorf solution were inoculated with the

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Aided by the John S. Swift Fund for Research in Asthma and Allied Conditions.

Presented, in part, before the annual meeting of the Association of Allergists for Mycological Investigations and the American Academy of Allergy, Feb. 5, 1956, St. Louis, Missouri.

Received for publication June 11, 1956.

viable cultures, after microscopic examination confirmed their purity. The flasks were kept at room temperature in the dark. They were agitated by shaking after incubation for ten days.

After a six- to ten-week growth, the mats were separated from the media by filtration through a sterile Buchner funnel and washed with sterile distilled water while still on the funnel. The large pieces of washed mats were further drained by wringing in a washed, sterile piece of cotton gauze. They were then chopped into small pieces with sterile scissors, placed in a sterile wide-mouth jar, and dried in a vacuum desiccator over calcium chloride at room temperature. When the mats were dry, they were enveloped in sterile paper and hammered into a fine powder. Microscopic examination of the mats, before desiccation, revealed spores and mycelia in the sporulating cultures and only mycelia in the nonsporulating cultures. The powder was defatted with commercial anhydrous ethyl ether (reagent grade) in a Soxhlet extraction apparatus. After first drying in the air and then in a vacuum desiccator over calcium chloride, the powder was then placed in a tightly stoppered container until extraction. Separate cultures of the powdered mats, both before and after defatting, resulted in a heavy growth of mycelia from the nonsporulating powder and a heavy growth of both spores and mycelia from the sporulating powder.

Extraction: Twenty per cent weight-by-volume extracts[6] of each mold were prepared by adding 50 ml. of 50 per cent glycerinated[7] Evans buffered saline[5] to each 10 grams of defatted powdered mold. Extraction was undertaken at refrigerator temperature (4° C.) for twenty-four hours, with frequent agitation by vigorous shaking. The insoluble residue was separated from the extracts by Buchner filtration or by centrifugation at 10,000 r.p.m. at 4° C., in a Servall Superspeed centrifuge. The extracts were sterilized by passage through a Seitz filter. The filtrates were transferred aseptically to sterile bottles. Sterility was ascertained by culture in Brewer's thioglycollate medium. The extracts were clear and of light amber color, and were labelled "WU sporulating #6-25-55" and "WU nonsporulating #5-23-55."

ANALYSES OF PM AND WU EXTRACTS

The phosphotungstic-acid-precipitable (PTA) nitrogen content of the four extracts was determined according to the method of Cooke and Stull,[4] as modified by Strauss and Spain.[13] Table I shows the protein nitrogen content (expressed in units*) of each extract.

TABLE I

EXTRACT	PROTEIN NITROGEN CONTENT (UNITS/ML.)
PM sporulating	39,000
WU sporulating	14,000
PM nonsporulating	35,000
WU nonsporulating	8,000

*One PNU = 0.00001 mg. PTA nitrogen.

During the experiments comparing the activity of these four extracts, all were diluted so as to contain the same concentration of protein nitrogen.

It is emphasized that different methods of extraction were used. Thus, the WU extracts were obtained from a one to five extraction of dry powdered mold mats, whereas the Prince-Morrow method[11] involved a one to fifty extraction of an especially prepared acetone precipitate from an original aqueous extract. The original ratio of extraction and total volume of original extract from which the PM acetone precipitates were prepared is not stated by Prince and associates.[10, 11, 15] Therefore, the true weight-volume extraction ratio of the original mold substances for the four extracts could not be calculated. The approximate weight by volume ratio of the dilution of 100 protein nitrogen units per milliliter (PNU/ml.) of each extract was estimated to be as shown in Table II.

TABLE II

EXTRACT	W/V RATIO (100 PNU/ML.)	ORIGINAL SUBSTANCE
PM sporulating	1-19,500	Acetone precipitate
WU sporulating	1-700	Dried, whole mat
PM nonsporulating	1-17,500	Acetone precipitate
WU nonsporulating	1-400	Dried, whole mat

The pH of each undiluted extract was determined and found to be 6.9 for both the PM and WU sporulating, 6.0 for the PM nonsporulating, and 6.4 for the WU nonsporulating preparations.

MEASUREMENT OF NEUTRALIZATION OF SKIN-SENSITIZING ANTIBODY

The cross-reactivity of the four extracts was determined by measuring their capacity to inhibit the reactivity of skin-sensitizing antibody (reagin) after the methods of Sherman and Stull[12] and Cooke,[2, 3] using in vitro mixtures of extract and human serum. Aliquots of a single dilution of a pool of thirty-six sera (from untreated patients) were mixed, in equal volumes, with each of the four extracts in the dilutions of 10, 50, 100, 200, and 400 protein nitrogen units per milliliter. Four to five hours after mixture, approximately 0.1 ml. of each mixture was injected intradermally into each of four sites, using the back of a nonclinically and nonskin-sensitive subject. About seventy-two to ninety-six hours later, each site was tested with approximately 0.02 ml. of each of the four appropriate extracts containing 1,000 PNU/ml. The immediate wheal reactions were evaluated about thirty minutes after testing. The lowest concentration of extract present in the serum-extract mixture which inhibited a positive reaction on subsequent retest was considered the end point of neutralization.

Fresh dilutions of both the serum and the extracts were made for each experiment. All extracts were diluted so that they contained equal protein nitrogen concentration. The same pooled reaginic serum was diluted one to eight for each experiment.

A pool of thirty-six sera, each containing skin-sensitizing antibody to alternaria extract, was prepared from samples obtained from thirty-six different untreated patients. Pooled sera were used during these neutralization studies to limit the possibility of the presence in the serum of a single dominant antibody to a particular antigen.

RESULTS

Fig. 1 illustrates one protocol of the neutralization tests. In the upper section is an experiment in which WU sporulating (WU-S) extract, in five different dilutions (10, 50, 100, 200, and 400 units per milliliter), was mixed with a one to eight dilution of the pool of sera. Seventy-two hours later each site was tested with the appropriate alternaria extract in the concentration of 1,000 units per milliliter. This particular extract neutralized well to itself (WU-S) and its corresponding nonsporulating (WU-NS) extract and also the two Prince-Morrow (PM-S and PM-NS) extracts. There was complete neutralization of the Prince-Morrow sporulating extract.

ALTERNARIA EXTRACT	CONCENTRATION OF RESPECTIVE EXTRACT MIXED WITH SERUM DILUTION (IN EQUAL VOLUMES) (PNU/ML.)	REACTIONS AT SITE WHEN TESTED AFTER 72 HOURS WITH 1,000 PNU/ML. OF:			
		WU-S	WU-NS	PM-S	PM-NS
WU sporulating	10	++	++	0	++
	50	0	0	0	0
	100	0	0	0	0
	200	0	0	0	0
	400	0	0	0	0
WU nonsporulating	10	++	+	+	++
	50	+	0	0	+
	100	+	0	0	+
	200	0	0	0	+
	400	0	0	0	+
PM sporulating	10	++	+++	+	++
	50	+	+++	0	+
	100	+	+++	0	+
	200	+	+	0	0
	400	+	+	0	0
PM nonsporulating	10	++	++	+	+
	50	+	++	0	+
	100	+	++	0	0
	200	+	+	0	0
	400	+	+	0	0
	Diluent	++++	++++	++++	++++

Fig. 1.—Passive transfer studies showing cross-neutralization reactions. Extracts of sporulating and nonsporulating *Alternaria tenuis*.
PNU = Phosphotungstic-acid-precipitable nitrogen units.
WU = Extracts prepared in the Washington University Laboratory.
PM = Extracts prepared in the laboratory of Drs. H. E. Prince and M. B. Morrow.

In the second section are presented the results when sites were prepared with the WU nonsporulating (WU-NS) extract and similarly tested. There was only moderate neutralization to WU sporulating (WU-S) extract, but strong neutralization to itself (WU-NS) and PM sporulating (PM-S) extract.

There was poor neutralization to PM nonsporulating (PM-NS) extract. These findings suggest that there is a difference in antigenicity between the WU and PM nonsporulating extracts.

From the experimental data presented in the third section, it can be seen that the PM sporulating (PM-S) was different antigenically from both WU extracts, as evidenced by the fact that there was no end point of neutralization. This extract did neutralize well to itself (PM-S) and it neutralized only moderately well to its nonsporulating counterpart (PM-NS).

Finally, the PM nonsporulating (PM-NS) extract failed to neutralize both WU extracts but neutralized well to itself and its corresponding sporulating extract (PM-S) (lowest section).

The findings in the lower two sections indicate that, with the particular pool of sera used, both of the PM extracts are similarly reactive, even though they are not capable of neutralizing the WU extracts prepared by different methods.

SERUM MIXED WITH ALTERNARIA EXTRACTS	SITES TESTED WITH ALTERNARIA EXTRACTS	DEGREE OF NEUTRALIZATION*					
		STRONG (<10 & 50)		MODERATE (100 & 200)		SLIGHT (400 & >400)	
WU sporulating	WU-S	x	xx	x			
	WU-NS		xx	xx			
	PM-S	xxxx					
	PM-NS	xx	x	x			
WU nonsporulating	WU-S		x	x		x	x
	WU-NS		xxx	x			
	PM-S	xx	xx				
	PM-NS				x		xxx
PM sporulating	WU-S		x			x	xx
	WU-NS				x	x	xx
	PM-S		xxx	x			
	PM-NS			x	xx	x	x
PM nonsporulating	WU-S		x			x	xx
	WU-NS				xx		xx
	PM-S	xx	xx				
	PM-NS	x	xx	xx			

Fig. 2.—Passive transfer studies showing cross-neutralization reactions. Extracts of sporulating and nonsporulating *Alternaria tenuis*.

*End point of neutralization in protein nitrogen (PTA) units per milliliter.

WU = Extracts prepared in the Washington University Laboratory.

PM = Extracts prepared in the laboratory of Drs. H. E. Prince and M. B. Morrow.

Fig. 2 summarizes the results of all the neutralization studies. All end points on all recipients are tabulated and are represented by "X"s. Each extract neutralized strongly to itself. In only two of four experiments, however, was the WU sporulating extract neutralized by small concentrations of its nonsporulating counterpart, whereas PM nonsporulating extract did neutralize to its sporulating counterpart. Neither PM extract, however, neutralized to either WU extract in the majority of instances; on the other hand, WU sporulating extract showed strong neutralizing capacity to all extracts.

It was evident that the WU and PM extracts neutralized to themselves, but cross-neutralization was not consistent.

COMMENTS

In 1942, Pratt[9] reported that the major antigenic activity was present in the extracts of spores rather than those of mycelia. The present study involved the use of extracts containing mycelia alone and extracts containing both mycelia and spores. Differences in methods of preparation of the mold extracts do not permit an absolute comparison of the results of the present study with Pratt's findings, since Pratt used extracts of spores alone and of mycelia alone. Furthermore, the method of preparation of both Washington University extracts differed greatly from that of the two Prince-Morrow extracts, making comparisons between the activity of the differently prepared extracts difficult. In the present study, results with the Washington University extracts tended to support Pratt's results to some extent, since the sporulating extract neutralized the nonsporulating to a much greater degree than the nonsporulating extract neutralized its sporulating counterpart. On the other hand, the Prince-Morrow nonsporulating extract did neutralize its corresponding sporulating extract.

Extracts of molds, like many other allergenic extracts, probably contain multiple antigens. Indeed, like other studies which compare activity of allergens prepared by different methods, the present work does not involve the use of a pure antigen.

Furthermore, it is known that the determination of phosphotungstic-acid-precipitable (PTA) nitrogen does not measure all the reactive components of an allergenic extract. It is reasonable to consider that, in the present experiments, the whole unfractionated extracts (WU) may contain components, active or inactive, which may not be present in the fractionated (PM) extracts. On the other hand, it is also reasonable to consider the possibility that the fractionated (PM) extracts may have been "purified" by such process and therefore contain more of the active components per unit volume.

It is known that identical extraction of different batches of the same raw material results in extracts often of widely different allergenic activity. The concentration of the PTA nitrogen gives an index of the relative activity, even though there are limitations to this method of standardization. Thus, the number of protein nitrogen units present in an extract may not be strictly comparable to the reactivity of the extract in the skin.

Limitations in the quantitative measurement of cross-reactivity of allergens by neutralization of skin-sensitizing antibody has been described in the literature. Pratt,[8] using fourteen different sera, showed that reciprocal neutralization between alternaria of three species was common but not invariable. Prince and his associates,[10] in their study on cross-reactivity of extracts of molds belonging to the Dematiaceae group, using individual nonpooled sera, found that differences in reactivity seemed related more to variations of reagins in the sera than to differences in mold extract. Sherman and Stull,[12] and also Cooke,[2, 3] have demonstrated that sera from individual patients vary greatly in their specificity. Their experiments showed that there was no constant relation between antigen, that there is no inherently dominant antigen,

and that each antigen is dominant only with reference to a particular serum. During studies on patterns of allergic sensitization, Wodehouse[14] found similar variation between individual sera and antigens. As mentioned previously, a pool of thirty-six different sera was used in the present study in order to lessen the likelihood of there being a dominant antibody for a particular antigen.

The possibility that the discrepancies in results were due to differences in the concentration of skin-sensitizing antibody, as measured by the serum dilution method, should be considered. The PM extracts showed negative reactions in a serum dilution of 1-32, while a dilution of 1-128 of the WU extracts was necessary before a negative reaction occurred (Fig. 3). This finding suggests that the pooled serum contained a greater quantity of skin-sensitizing antibody (reagin) for the WU extracts than for the PM extracts.

SERUM DILUTION (POOL #55-119)	REACTION ON TEST WITH ALTERNARIA EXTRACT 1,000 PNU/ML.			
	WU-S	WU-NS	PM-S	PM-NS
1-1024	0	0	0	0
1-512	0	0	0	0
1-256	0	0	0	0
1-128	0	0	0	0
1-64	±	+	0	0
1-32	+	+	0	0
1-16	++	++	++	++

Fig. 3.—Titration of skin-sensitizing antibody.
PNU = Phosphotungstic-acid-precipitable nitrogen units.
WU = Extracts prepared in the Washington University Laboratory.
PM = Extracts prepared in the laboratory of Drs. H. E. Prince and M. B. Morrow.

However, the serum dilution method for titrating reagin may not indicate the true quantity of skin-sensitizing antibody present in serum. The results of the cross-neutralization studies are not sufficiently consistent to permit the conclusion that this phenomenon would explain the failure for universal cross-neutralization with the four mold extracts. The pooled serum contained more reagin to the WU extracts than to the PM extracts; yet WU sporulating extract neutralized well to all extracts. However, both PM extracts neuralized only slightly to both WU extracts, even though there was less reagin to the PM extracts. It would have been desirable to have sera which had the same skin-sensitizing titer to each of the four extracts.

Since it has been shown that glycerin in the concentration of 50 per cent preserves the activity of ragweed and timothy pollen extracts[6, 7] and since each alternaria extract contained 50 per cent glycerin, the fact that the PM extracts were 4 months older than the WU extracts at the time of these comparative studies probably does not account for differences in activity.

Nevertheless, the previously mentioned differences in the method of preparation of the two sets of extracts as well as the differences in the concentration of reagin to each extract should be considered in the interpretation of these findings. This work compares, by the methods used, four different extracts of alternaria. It is not the purpose of this article to demonstrate whether any particular extract or any particular method of extraction is superior.

Further studies, using extracts prepared from both sporulating and nonsporulating cultures derived from the same original culture, might offer more clear-cut determination of whether or not mycelia as well as spores contained the pertinent antigen or antigens. Perhaps such could be accomplished by special methods of culture.

SUMMARY

1. Extracts of sporulating and nonsporulating *Alternaria tenuis* were prepared in two different laboratories by two different methods. One set was supplied through the courtesy of Drs. Homer E. Prince and Marie B. Morrow who had prepared them, by a special procedure, from an acetone precipitate derived from an aqueous extract of whole mold mat. The other set of extracts was prepared by direct extraction of whole mold mats in the laboratory of the Immunology Division of the Washington University School of Medicine.

2. The four alternaria extracts, after dilution to equal concentration of protein nitrogen, were studied for allergenic activity and cross-reactivity by the quantitative measurement of neutralization of the skin-sensitizing antibody of a pool of thirty-six human sera, each containing reagin to alternaria extract.

3. Each extract was titrated simultaneously against itself and against the other three extracts. The results which indicated significant neutralization of skin-sensitizing antibody were:

(a) Only the WU (Washington University) sporulating extract neutralized to all four extracts.

(b) The WU nonsporulating extract neutralized to itself and to PM (Prince-Morrow) sporulating extract, but not to the other two extracts.

(c) The PM sporulating extract neutralized only to itself.

(d) The PM nonsporulating extract neutralized to itself and to its sporulating counterpart, but to neither of the WU extracts.

Thus, each of the four extracts neutralized to itself, but there was no consistent pattern of cross-neutralization.

ADDENDUM

Since the presentation of this paper, an article by Prince and associates[15] was published. The authors compared the reactivity of solutions of acetone precipitates of extracts of sporulating and nonsporulating *Alternaria tenuis*, using direct skin tests and in vivo neutralization tests, and found that both extracts behaved similarly.

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AEROBIC BACTERIA IN HOUSE DUST

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THE present study deals with the aerobic bacteria in house dust. Numerous studies in the various constituents of house dust have been made since Cooke's[1] paper in 1922, in which he described house dust as a cause of symptoms in patients with rhinitis and asthma. Previous studies[2, 3] on the bacterial flora of house dust have been concerned in the main with that from specialized locations, such as hospitals, and have dealt with house dust as a cause of infection. Little investigation has been carried out on the bacterial content of dust collected from within dwellings as a part of the over-all study of the constituents of house dust as an allergen in the causation of hypersensitivity.

In the present study, bacteriologic investigations were carried out on weighed aliquots of thirty-eight samples of house dust gathered from vacuum cleaners used in houses and apartments in the New York area. Most of the samples came from the homes of asthmatic patients in the New York Hospital Allergy Clinic.

METHOD

In our study, 200 mg. portions of dust were incubated at 37° C. for five hours in 50 c.c. beef extract. A loopful of the broth culture was streaked on blood agar and cultured aerobically. A quantitative approximation of the various colonies was made from the twenty-four-hour plates. Colonies were

TABLE I. DEFINITIONS OF SEVEN BACTERIAL FAMILIES ISOLATED FROM THIRTY-EIGHT DUST SAMPLES

FAMILY	DEFINITION
Bacillaceae	Spore-forming rods
Corynebacteriaceae	Diphtheroids
Enterobacteriaceae	Coliform group
Bacteriaceae	Heterogenous collection of nonspore-forming rods whose relationships to each other and to other groups are not clear
Micrococcaceae	Staphylococci and cocci occurring in groups, never in chains
Neisseriaceae	Gram-negative cocci
Lactobacteriaceae	Gram-positive cocci in chains

isolated and differentiated morphologically and biochemically,[4] and were classified according to families (Table I), using whichever of the following criteria were applicable: motility; Neisser stain for metachromatic granules; Huntoon's stain for spores; Gram's stain; capsule stain; fermentation of dextrose;

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Received for publication Nov. 26, 1956.

lactose, sucrose, maltose, and mannitol; indole production; nitrate reduction; action on bromcresol purple milk; utilization of $NH_4H_2PO_4$ as sole source of nitrogen; gelatin liquefaction; production of acetylmethyl carbinol; methyl red test; coagulase production; production of NH_3 from peptone; and general colony morphology. Animal inoculations for pathogenicity were not performed.

QUALITATIVE STUDIES

With the exception of the *Micrococcus pyogenes* species in the Micrococcaceae family, the great majority of bacteria isolated were obscure strains unidentifiable beyond family designation. Results of the bacterial analysis are summarized in Table II. Because of our special interest[5] in the *Staphylococcus*

TABLE II. BACTERIAL ANALYSIS OF THIRTY-EIGHT SAMPLES OF HOUSE DUST SHOWING FAMILIES PRESENT AND COLONY COUNTS PER SAMPLE

COLONY COUNT	A	B	C	D	E	F	G	H	I
Over 50	3	1	0	2	4	0	0	0	0
25 to 50	9	1	2	0	7	1	0	1	1
10 to 25	7	8	3	4	9	0	0	0	0
1 to 10	19	7	5	16	12	3	1	3	5
Total	38	17	10	22	32	4	1	4	6

A = Bacillaceae.
B = Corynebacteriaceae.
C = Enterobacteriaceae.
D = Bacteriaceae.
E = Micrococcaceae.
F = Neisseriaceae.
G = Lactobacteriaceae.
H = *M. pyogenes var. aureus.*
I = *M. pyogenes var. albus.*

(Micrococcus pyogenes), we investigated this group more intensively. The Bacillaceae were present in 100 per cent of the samples, Micrococcaceae in 89 per cent, Bacteriaceae in 58 per cent, Corynebacteriaceae in 45 per cent, Enterobacteriaceae in 26 per cent, Neisseriaceae in 11 per cent, and Lactobacteriaceae in 3 per cent. The *M. pyogenes* species is relatively inconspicuous. A large proportion of the samples showed light growth (one to ten colonies per sample) but none were sterile. The number of bacterial families per sample of dust ranged from one to five, with a mean of 3.3. In seven samples one organism predominated and in ten samples two to four organisms were equally

TABLE III. QUANTITATIVE DETERMINATION OF AEROBIC BACTERIA IN TEN SAMPLES OF HOUSE DUST

SAMPLE	AVERAGE COLONIES PER GRAM
D-9	389,000
D-10	970,500
D-14	4,038,000
D-17	29,000
D-19	730,000
D-22	3,440,000
D-26	1,130,000
D-30	1,880,000
D-35	2,402,000
D-48	3,012,000

abundant, whereas thirteen samples contained from two to five organisms in equally meager amounts. In order to check the admittedly empirical culture method, we cultured eight of the dust samples a second time, and these duplicate samples paralleled the original results.

QUANTITATIVE STUDIES

Bacterial counts on ten samples were determined, using forty-eight-hour agar pour plates prepared from sterile saline suspensions of 1.0 Gm. portions of dust. Total counts in ten samples varied widely, ranging from 29,000 to 4,038,000 colonies per gram, with an average of 1,802,850 colonies per gram (Table III).

DISCUSSION

The maximum number of aerobic bacteria found per gram of dust was about 4 million. The maximum weight of live aerobic bacteria per gram of house dust would be about 5 mg. This is so small a portion (0.5 per cent) that, in all probability, the amount of live aerobic bacteria contained in one skin test dose of house dust extract is negligible. This, of course, does not eliminate the possibility that dead bacteria or bacterial breakdown products may be involved in house dust allergy.

SUMMARY

Bacteriologic study of thirty-eight samples of house dust collected in the New York area have revealed a flora which varies widely, qualitatively and quantitatively. Representatives of seven aerobic bacterial families were observed, only one of which, Bacillaceae, was consistently present. As determined by accepted biochemical tests, the bacteria appeared to be chiefly nonpathogenic forms.

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THE ASTHMATIC SYNDROME PRECIPITATED BY FOREIGN BODIES IN THE LUNG

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THE following two case reports emphasize two points: (1) the difficulty in demonstrating a foreign body in the lung, even though an adequate history is obtained, and (2) the close similarity between asthmatic wheezing precipitated by foreign bodies and that caused by conventional allergens.

Case 1.—C. W., a 45-year-old Negro woman, presented herself at the Parkland Hospital on Aug. 4, 1949, at 4:30 P.M., with a history of having eaten chicken at the noon hour and of possibly having swallowed a chicken bone. She was coughing and had questionable difficulty in swallowing. There was no antecedent history of asthma or any chronic chest disease.

A chest x-ray film was made on the same date, and it was reported that there was no roentgenographic evidence of a radiopaque foreign body superimposed over the esophagus or trachea.

On Aug. 6, 1949, two days later, she was bronchoscoped with a 7-40 bronchoscope. The report stated: "The coryna was seen to be sharp and located in the midline. The scope was then carried down into the right main bronchus. The orifices to the main and middle lobes were seen and no foreign body was visualized. The upper right lobe orifice could be seen in a fleeting glimpse. The scope was withdrawn and introduced into the left main stem bronchus. A marked amount of purulent secretion was encountered. It was removed with suction, and two small nodules measuring approximately 2 cm. in diameter were seen. They were pearly white and glistening. A biopsy specimen was sent to the laboratory." The microscopic examination of this specimen revealed it to be a small amount of bronchial mucosa and unidentified necrotic tissue.

This patient was again admitted to the Parkland Hospital on Aug. 25, 1949, for further studies. At this time, she had a mild cough and an occasional wheeze. No hemoptysis, pain, or loss of weight was noted. She was hospitalized primarily for diagnostic studies, rather than for palliative treatment of symptoms. On Aug. 29, 1949, posteroanterior and lateral chest films showed no evidence of pneumonia, tuberculosis, or other parenchymatous disease involving either lung field. Lateral views of the cervical and upper thoracic regions for visualization of the trachea showed a 2 to 3 mm. change in diameter of the trachea during the respiratory cycle. There was no roentgenologic evidence of an extrinsic mass causing pressure on the trachea. Following x-ray studies, a second bronchoscopy was reported: "The false and true cords both appeared normal. The tracheal mucosa appeared normal. The coryna was sharp and situated in the midline. In the left main stem bronchus, hyperemic bronchial mucosa was seen overlying the orifice to the left lower lobe. The orifice to the lower lobe and the lingula appeared to be normal. Secretions were aspirated from this area for Papanicolaou stain. The right side appeared normal." There was no improvement in her symptoms following this procedure.

Presented at the twelfth annual meeting of The American Academy of Allergy, St. Louis, Missouri, Feb. 5 to 7, 1956.

Received for publication Aug. 2, 1956.

On Sept. 15, 1949, fluoroscopic and film studies of the bronchi with Lipiodol instillation showed the Lipiodol to enter the left main stem bronchus without obstruction and traverse the bronchi to the left upper and lower lobes of the lung without obstruction or roentgenologic evidence of defect.

The patient remained in the hospital from Aug. 25 to Sept. 21, 1949, and during this time she developed intermittent episodes of asthmatic wheezing with evanescent bilateral râles over the entire chest field which responded to the usual bronchodilating medications. The asthma was not severe, but râles could be heard without the stethoscope at the open mouth of the patient. Eventually, this patient was discharged with the diagnosis of allergic laryngitis, etiology undetermined, and asthmatic wheeze, etiology unknown. In the allergy clinic, on Sept. 21, 1949, bilateral asthmatic râles were heard over the entire chest field but there was no evidence of a predominance of wheezing in any one lobe or group of lobes.

For the next eighteen months, this patient was studied for possible allergic etiology of her disease. Equivocal skin test reactions were obtained to ragweed, feathers, house dust, and flaxseed, and the patient was treated with no improvement. Elimination diets achieved no benefit. Elimination of suspected environmental antigens offered no help. Her asthma was controlled satisfactorily with Adrenalin, ephedrine, and aminophylline. She was seen approximately every week during these eighteen months. On some occasions she would be wheezing, with no localized signs found. At other times, she would have a perfectly clear chest. The wheezing came and went without rhyme or reason, so far as she was concerned. She had no hemoptysis, weight loss, or pain. A close relative died of malignancy in a paranasal sinus, and this caused the patient a considerable amount of alarm with regard to herself. On two occasions, she stated that she had flecks of blood which came out of the nasopharynx, or at least she thought that it came from this region and not from the lung. Physical and roentgenologic examinations did not reveal any abnormality in this region.

After approximately eighteen months of treatment in the allergy clinic, she stopped coming. Approximately two months later, on May 23, 1951, she developed fever, cough, and dyspnea, and went to the emergency room for immediate attention. An x-ray picture was made of her chest, which showed a 4 cm. area of poorly defined homogeneous consolidation in the left lateral sulcus, adjacent to the diaphragm. The rest of the lung field was clear. The conclusion drawn from this examination by the roentgenologist indicated acute inflammation in the dorsal basilar segment of the lower left lobe. Penicillin was given. On May 27, 1951, the patient reported again to the emergency room, where she received more penicillin. She complained of pain in her shoulder, and was referred to the orthopedic clinic. Subsequently, she returned to the emergency room where, on June 13, 1951, another x-ray examination was made which showed that she had an area of pulmonary consolidation in the left costophrenic sulcus, with a small amount of left pleural effusion. The statement was made that the roentgenologist could not rule out a bronchogenic carcinoma. She was then referred to the general medicine clinic, where she had a complete diagnostic work-up, and the conclusion of the consultant was: "Apparently a problem of bronchial asthma, with superimposed pulmonary infection." She was treated with potassium iodide, and then released from the medical clinic.

Subsequently, this patient decided of her own volition to return to the allergy clinic in August of 1951, and she stated once again that she was having asthma and trouble with her lungs. A review of the patient's chart revealed the episodes of pneumonia that she had had in May and June and the treatment in the emergency room. This, of course, was a clue to the probable diagnosis—obstruction to the bronchus of the left lower lobe. Once again she was x-rayed and an inflammatory consolidation in the left base of the lung was found.

The patient was referred again to the chest service for bronchoscopy. The mucous membrane of the left main bronchus was definitely inflamed and there was an object, which appeared to be a foreign body, situated in the left main bronchus, about 1 cm. from the coryna. This was removed on Sept. 26, 1951, and was shown to be a piece of bone.

The patient did not report to the allergy clinic after bronchoscopy. After a lapse of twenty-seven months, the patient's daughter, the patient's employer, and the patient were consulted separately (without either of them knowing that the others had been consulted), with regard to the patient's condition following removal of the foreign body. All three agreed that the patient had a dramatic cure of the so-called asthma. This case suggests the following conclusions:

1. It is difficult at times to demonstrate a foreign body in the tracheal or bronchial tree. Several bronchoscopists were unable to locate the suspected foreign body.

2. A foreign body in one side of the lung can produce bilateral râles indistinguishable from those produced by bronchial asthma.

3. The râles were not constant but were evanescent, just as they are in any patient with allergic bronchial asthma. She felt perfectly well on some days; at other times, she had definite, clear-cut attacks of wheezing.

4. This patient stated that she was nervous in the past, as well as at the present time, and suspected that this might be playing a role in the etiology of the ashma, since she had been convinced that there was no foreign body in her lung after the long, careful work-up she had received at the onset of the illness. She definitely did develop cancerphobia. It is quite difficult, at times, to determine in any patient whether nervous manifestations are the cause or the result of an illness. This patient was definitely placed in the latter category.

5. She responded to the usual symptomatic remedies for asthma.

6. It was twenty months from the aspiration of the foreign body into the bronchus before she developed evidence of pneumonia.

7. The two episodes of pneumonia in the same lobe suggested the diagnosis in this case and the physicians were then able to direct the bronchoscopist's attention toward the most probable location of the foreign body.

Case 2.—K. F., a 13-month-old boy, was born into a family in which an older brother, aged 13, had typical asthma. The parents were fully aware of the history of an asthmatic child. The patient was cared for by a pediatrician, who had noted that colds were accompanied by some asthmatic wheezing. In view of the over-all picture, the pediatrician felt that this child was developing bronchial asthma. In May, 1952, when the boy was 13 months old, the maid took him for a stroll. She noticed that very suddenly he developed a severe cough and she thought that he had picked up a rock or some other object and swallowed it. His mother heard this severe coughing and ran to him, turned him upside down, and struck him a few times, after which a stone dropped out of his mouth. He was not cyanotic but was wheezing at this time. She then took him to a chest surgeon who did a physical examination and made a rogentgenogram of the chest, but found nothing that seemed to indicate a foreign body in the air passages. The wheezing continued day and night for the next two months. A hypodermic injection of Adrenalin relieved the child very little, or none at all. On June 9, 1952, the chest surgeon sent him to the allergy clinic where a careful allergy study was done to see if the etiology of his wheeze could be determined. The patient had just finished the examination when his mother took him to Arizona. After two weeks without improvement, she brought the child back to the allergy clinic. At this time, physical examination revealed râles over the entire lung field. They did not seem to be fine musical râles, but were of a coarse nature. There was questionable evidence of the presence of a stridor. This child received no benefit from intramuscular epinephrine and rectal suppositories of aminophylline.

At this point, the chest surgeon did a bronchoscopy and found a piece of hard tinfoil in the right main stem bronchus. Ever since the removal of this foreign body, this child has remained perfectly well; he has had no more attacks of asthma in the four years that have followed.

This second case demonstrates very clearly that it is possible for a metallic foreign body to be present in the lung without it showing on the x-ray film. Careful x-ray studies are needed in situations of this type, with different techniques used to study objects of different densities. Also, when a youngster develops sudden onset of intractable wheezing without any obvious explanation and does not respond to the usual drugs, a foreign body should be suspected until the possibility is proved or disproved.

SUMMARY

Two cases of an asthmatic syndrome produced by a foreign body in the bronchus are presented. A perusal of the literature would suggest that foreign bodies of the lung are relatively easily demonstrated if one suspects their presence and obtains x-rays. In the two cases presented, the referring physician did suspect a foreign body and secured consultation from a radiologist. Confirmatory proof could not be demonstrated by means of roentgenograms. In one case, the first two bronchoscopic examinations were negative. Both patients had bilateral râles; these were constantly present in one and intermittently present in the other.

Announcements

The Jewish Hospital of Brooklyn

Clinical Assistant

Openings for clinical assistants are available in the Allergy Clinic of The Jewish Hospital of Brooklyn. Members of the clinic staff attend clinical conferences, seminars, and literature review meetings. The opportunities for self-development in the field of practical allergy and experimental investigation are abundant. There will be an opportunity for advancement to the inpatient service.

Those interested are requested to contact Dr. Max Grolnick, attending in allergy at The Jewish Hospital of Brooklyn.

Residency in Allergy

The Allergy Division of The Jewish Hospital of Brooklyn will have an opening for a resident in allergy beginning July 1, 1957.

The opportunities for development are numerous. The department is an active one, affording a wide scope of both detailed clinical study and basic experimental investigation. Upon completion of the residency, an inpatient staff appointment will be offered, if the obligations of the position can be fulfilled.

Those interested are requested to contact Dr. Max Grolnick, attending in allergy at The Jewish Hospital of Brooklyn.

Sociedad Argentina de Alergia

The new officers of the Sociedad Argentina de Alergia for 1957-1958 are:

President: Dr. Miguel Agustin Solari
Vice-President: Dr. Jose F. Dumm
Secretary: Dr. Andres Martinez Marchetti
Assistant Secretary: Dr. Lorenzo Giscafre
Treasurer: Dr. Heriberto Minsk
Assistant Treasurer: Dr. Salvador Pisani
Associate Members: Dr. Guido Ruiz Moreno
Dr. Caupolican Castilla
Dr. Jose Martorelli
Dr. Leon Bentolila
Dr. Osvaldo Crivelli

Book Reviews

Dermatology. By Donald M. Pillsbury, Walter B. Shelley, and Albert M. Kligman. Philadelphia and London, 1956, W. B. Saunders Company. 1331 pages, 564 figures. Price, $20.00.

Diseases of the Skin. By Richard L. Sutton. Eleventh edition. St. Louis, 1956, The C. V. Mosby Company. 1479 pages, 1972 figures. Price, $29.50.

Practical Pediatric Dermatology. By Morris Leider. St. Louis, 1956, The C. V. Mosby Company. 433 pages, 293 illustrations. Price, $10.50.

Clinical Selections in Dermatology and Mycology. By Frederick R. Schmidt. Springfield, Illinois, 1956, Charles C Thomas, publisher. 505 pages. Price, $10.50.

Atlas und Prakticum der Dermatologie und Venerologie. By W. Burckhardt. München and Berlin, 1955, Urban and Schwarzenberg. 279 pages, 158 illustrations (99 in color). Price, $11.50.

Most allergists approach their specialty through training in internal medicine or pediatrics, often with little or no experience in dermatology. Yet in their practice they are faced with a variety of skin diseases mistakenly diagnosed as allergic, and not infrequently will find that both patients and some medical colleagues confuse allergists with dermatologists. Furthermore, in the treatment of allergic dermatoses a knowledge of the general principles of dermatology is a necessary adjunct to the purely immunologic approach. For all these reasons, reference books on dermatology are frequently needed. This year has brought a number of useful publications in this field.

Both the eleventh edition of Sutton's well-known book and the new volume by Pillsbury, Shelley, and Kligman are complete expositions of the subject, and serve as adequate references for most purposes. Sutton's book is remarkable for its large number of excellent black and white illustrations and for many references to the literature. The first four sections of Pillsbury, which cover the basic principles of dermatologic diagnosis and treatment, are particularly instructive to those without training in this field. Both books minimize the value of the allergic approach in infantile ezcema and atopic dermatitis; many physicians interested in their approach will feel that their views are overly skeptical in this regard.

Leider's book presents, in a far briefer form, the diagnosis and treatment of skin disorders of infants and children, and should prove useful for the allergist who limits his practice to this age group.

Schmidt's book, which contains contributions by thirty-six specialists throughout North and South America, is quite different in character from those previously described. Although most aspects of dermatology receive attention, it remains a collection of specialized articles rather than a systematic coverage of the whole subject. Controversial opinions and original ideas of the contributors are purposely emphasized, often to the neglect of well-known basic factors. This handling of the subject is more suitable for the well-trained and critical dermatologist than for physicians in other fields of medicine. The average allergist will find it less useful for reference, particularly with regard to differential diagnosis.

Burckhardt's book is primarily an atlas of the common skin diseases, and is recommended for its excellent color plates. The text (in German) is brief.

Who Is Who in Allergology. (Published under the auspices of the International Association of Allergology). By Ejnar Munksgaard, Copenhagen; edited by Egon Bruun, M.D., Copenhagen.

Dr. Bruun has put together the lists of the members of every allergy society in the world, with a virtual *curriculum vitae* of each man, including a list of his publications with titles and usually with the bibliographic reference.

In the world, there is a total of forty-seven allergy societies in twenty-eight countries. Membership varies from 5 members of La Sociedad Colombiana de Alergia to over 1,000 members of the American College of Allergy.

The book is presented in loose-leaf form, so that new pages can be inserted to make it a living compilation. The editor says, in his preface: "This first edition is to be considered as a draft. . . . It is hoped that each member of each society will appreciate the fundamental idea and collaborate in the future, so that the purpose may be fulfilled."

In future editions, one would like to see more facts and figures included in the preface, with comments on the present status of clinical allergy in the different countries. For example, there are only three countries which have more than one allergy society: Argentina and Australia each has two and the United States has eighteen. Altogether there are forty-seven allergy societies, with a total of 3,135 members.

On the page before the list of members of each society, a brief history of that society should be presented. When was it formed? Who was the original moving spirit? How often and where are the meetings held? Who are the present officers, and what is the secretary's address? Information like this would make the book interesting as well as useful.

The present format is necessary if new sections are to be inserted in the future, but this loose-leaf system makes the binding large and clumsy. Each country is marked by a sheet of heavy paper. This makes for easy finding, but the heavy paper increases the bulk and weight enormously. To leave out these heavy papers would leave 644 pages of text, which is not too excessive. If the name of the country could be printed in the right upper corner of each page, there should be little difficulty in finding it.

This compilation is very useful. Every medical library should have a copy, and all persons who are lucky enough to have contacts with fellow workers in other areas will find the book almost essential. For doctors whose patients are about to travel in foreign countries, the book will be very helpful in finding the names of those doctors who can be called in case difficulties or emergencies should arise.

As Dr. Wittich, Past-President of the International Association of Allergology, writes in his foreword: "It required a young physician of international reputation in the field of allergy, both as a successful clinician and investigator, to undertake this great task." As one might expect, he did it well.

F. M. R.

Obituaries

CONRADO B. ICASIANO died at the age of 49 in the Walter Reed Hospital on Feb. 29, 1956. Dr. Icasiano was graduated from the College of Medicine, University of the Philippines, in 1939. After graduation, he served as resident in the Philippine General Hospital, Manila. In 1937 he was commissioned in the Medical Corps, Armed Forces of the Philippines, and at the time of his death he was a Lieutenant-Colonel and Chief of the Medical Services of the Victoriano Luna General Hospital, Queson City. He was a member of the Philippine Medical Association, the American Diabetic Association, and the American Academy of Allergy.

LUTHER J. KING died on Aug. 29, 1956. He was born in 1896 and graduated from the University of Pennsylvania in 1922. From 1931 to 1945 he was on the staff of Spenser Hospital, Meadville, Pennsylvania, as a pediatrician. He was a member of the Crawford County and Pennsylvania State Medical Societies, and was also a member of the International Correspondence Society of Allergists. He had been a member of the American Academy of Allergy since 1947.

JOHN P. PARSONS of Grosse Point Park, Michigan, died last September. He was born in 1889, and graduated from the University of Michigan Medical School in 1919. During World War I he served as a private. He was a fellow of the American Medical Association, the American Academy of Allergy, and the American Academy of Pediatrics. He was also a member of the American Association for the Advancement of Science.

AMADAEO VINCENTE-MASTELLARI died on Sept. 9, 1956, after a prolonged illness. He died of uremia secondary to malignant nephrosclerosis and malignant hypertension.

At the time of his death, Dr. Mastellari was chief of the Chest Service, Gorgas Hospital, Ancon, Canal Zone, professor of phthisiology at the School

of Medicine, National University of the Republic of Panama, and director of the Anti-Tuberculosis Campaign of the Republic of Panama. He belonged to the following associations:

Fellow of The American College of Physicians (life member) and Governor for Central America and Panama.

Fellow of The American College of Chest Physicians and Regent for Central America.

Fellow of The American Public Health Association.

Fellow of The American Trudeau Society.

President of The Isthmian Medical Association of the Canal Zone.

Assistant Editor of the *Journal of Chest Diseases.*

In addition to these, Dr. Mastellari belonged to numerous medical societies in both South and North America, especially those interested in diseases of the chest.

CHARLES W. WARREN died on Nov. 4, 1956, in Rochester, New York, after a coronary occlusion. He was 56 years old. Dr. Warren was a graduate of the Syracuse University Medical College. He was a fellow of the American Academy of Allergy and of the American College of Physicians and a member of the American Diabetic Association. He was a diplomate of the Board of Internal Medicine at the School of Medicine and Dentistry of the University of Rochester and an assistant attending physician at the Strong Memorial and Rochester Municipal Hospitals.